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Diagnostic and predictive biomarkers for superimposed pre-eclampsia

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Abstract

Women with chronic kidney disease (CKD) and chronic hypertension (CHT) frequently develop superimposed pre-eclampsia but distinction from pre-existing disease is challenging. Plasma placental growth factor (PlGF), B-type natriuretic peptide (BNP), neutrophil gelatinase-associated lipocalin (NGAL) and serum relaxin concentrations were quantified in a longitudinal prospective cohort of women with CKD (n=121), CHT (n=44) and healthy controls (n=79) and biomarker concentrations compared with women with pre-eclampsia without pre-existing disease (n=32). Test performance was evaluated for diagnosis of superimposed pre-eclampsia requiring delivery within 14 days of sampling. PlGF was evaluated as a promising marker in a validation cohort in a validation cohort of women with suspected pre-eclampsia (CKD n=29; CHT n=94; superimposed pre-eclampsia requiring delivery within 14 days n=29) and compared to women without pre-existing disease (No pre-eclampsia n=290; pre-eclampsia requiring delivery within 14 days n= 176). Between 20^{+0}-36^{+6} weeks' gestation, PlGF had high diagnostic accuracy for superimposed pre-eclampsia requiring delivery within 14 days (receiver operator characteristic [ROC] 0.85; SE 0.06) and was confirmed (ROC 0.82; SE 0.06) in the validation cohort. Plasma NGAL, BNP and serum relaxin concentrations were not discriminatory for superimposed pre-eclampsia. A low maternal plasma PlGF concentration could be a useful adjunct to guide decisions regarding admission and delivery for superimposed pre-eclampsia.

Key Words: Chronic kidney disease; Endothelium; Proteinuria

Introduction

Pre-eclampsia is estimated to complicate 2-8% of all pregnancies;[1] however superimposed pre-eclampsia is reported to affect approximately 26% of pregnant women with chronic hypertension (CHT)[2] and 22-75% of women with chronic kidney disease (CKD).[3] The diagnosis of pre-eclampsia when superimposed upon chronic kidney disease (CKD) and chronic hypertension (CHT) is challenging as it may be clinically indistinguishable from benign gestational progression of pre-existing hypertension and proteinuria, which often coexist.[4] Superimposed pre-eclampsia is frequently associated with poor maternal and fetal outcomes. Therefore early and accurate diagnosis is essential to allow timely intervention, whilst misdiagnosis may also lead to unnecessary admissions and iatrogenic preterm delivery.

Current hypotheses describe abnormal placental perfusion and predisposing maternal factors[5] (including cardiac, vascular and renal dysfunction) in the genesis of pre-eclampsia, but the relevant contribution of placental and maternal influences to development of superimposed pre-eclampsia is poorly understood. The aims of this study were to evaluate the predictive and diagnostic performance for superimposed pre-eclampsia of markers of placental, cardiac and renal function which have previously been implicated in pre-eclampsia in women without underlying disease.

The markers studied were placental growth factor (PlGF), soluble fms-like tyrosine kinase receptor (sFlt-1), B type natriuretic peptide (BNP), neutrophil gelatinase associated lipocalin (NGAL), and relaxin. PlGF, an angiogenic protein synthesised by syncytiotrophoblasts increases in the blood of healthy pregnant women until 26-30 weeks and then fall towards term[6]; low plasma PlGF has been reported in women with pre-eclampsia.[7] Conversely sFlt-1, an anti-angiogenic protein which binds to PlGF preventing interaction with endothelial receptors is raised in women with pre-eclampsia.[8] Higher than normal concentrations of BNP[9, 10] are observed in women with pre-eclampsia; BNP is released with cardiac ventricular strain, and is raised prior to diagnosis in women with early onset pre-eclampsia.[11] NGAL , an early marker of acute kidney injury (AKI) has been associated with pre-eclampsia in women without pre-existing disease[12,13] and relaxin is an ovarian hormone known to play an important physiological role in the renal adaptation to pregnancy.[14]

Measurement of these biomarkers was undertaken in four groups of women with CKD and/or CHT with superimposed pre-eclampsia; CKD and/or CHT without superimposed pre-eclampsia; pre-eclampsia without CKD and/or CHT and healthy controls. We validated the best performing marker in a second cohort of women with suspected pre-eclampsia or superimposed pre-eclampsia. The primary outcome of superimposed pre-eclampsia requiring delivery within 14 days was chosen to provide a clinically relevant endpoint that reflects current management strategies.[15]

Results

Longitudinal Cohort

288 women were recruited to the study (Figure 1a). Of those consented, 11 were lost to follow-up and one was subsequently recruited to a treatment trial for pre-eclampsia. The remaining 276 women provided 471 samples for analysis.

Demographics

Baseline demographic data are presented in Table 1. Details of underlying disease in women with CKD and/ or CHT are shown in Supplementary Table 2. There were no demographic differences between women with CKD and/or CHT who did or did not develop superimposed pre-eclampsia.

Demographics for women with CKD and/or CHT according to severity of CKD are shown in Supplementary Table 3. Twenty-five (21.6%) of 121 women with CKD had the diagnosis of CKD made during the index pregnancy, including six (26%) of the 23 women with CKD who subsequently developed superimposed pre-eclampsia.

Maternal outcomes

Maternal outcomes are shown in Table 2. Forty women (24.2%) were diagnosed with superimposed pre-eclampsia. Twenty-three women with CKD (19.0%) developed superimposed pre-eclampsia, including 8/61 women (13.1%) without secondary CHT, and 15/45 (37.5%) with CHT (P=0.1)), whereas 12/39 women (30.8%) with primary hypertension developed superimposed pre-eclampsia (excluding 5 women who were recruited at time of disease). Only eight women with superimposed pre-eclampsia (20%) had elevated alanine transaminase (ALT) or low platelets.

Women with superimposed pre-eclampsia were delivered for indications related to its development including maternal hypertension (n=15; 37.5%), deteriorating renal function (6; 15%), liver abnormalities (2; 5%), neurological symptoms (2; 5%), fetal indications (10; 25%) and post 37 weeks' gestation (2; 5%) and the indication was not clear in two cases (2; 5%). Forty-four (45%) of 98 women with CKD without superimposed pre-eclampsia had iatrogenic delivery for reasons including 'deteriorating renal function' (12; 27%), increasing proteinuria (2; 4.5%), increasing hypertension (2; 4.5%) and the presence of stable CKD (4; 9.0%). Women with CKD and/or CHT had more antenatal admissions than healthy controls (P<0.0001). Women with superimposed pre-eclampsia were also more likely to have longer peripartum admissions than women with those without (p<0.0001), but did not have any more antenatal admissions.

Acute kidney injury

Overall, 29 (24.2%) women with CKD and 10 (22.7%) women with CHT developed pregnancy associated sub-acute kidney injury. Women with CKD who developed acute kidney injury, pregnancy associated sub-acute kidney injury or postpartum progression of renal disease including those who also met study criteria for superimposed pre-eclampsia are described in Table 3. None of the women required renal replacement therapy either during or after pregnancy, except one woman already receiving haemodialysis, and there were no maternal deaths.

Neonatal outcomes

Neonatal outcomes are shown in Table 4. Neonatal outcomes according to underlying presence of CHT and CKD stage are shown in Supplementary Table 4.

PlGF changes with gestation

Figure 2 shows the gestational profile of PlGF concentrations in 471 samples from 276 women. Following interval regression (46 left-censored observations, one right-censored) adjusted for gestation at sampling, women with pre-eclampsia without pre-existing disease had lower PlGF concentrations ($p<0.001$) and Z- scores than healthy controls ($p<0.001$) at all gestational time points. Women with CKD and/or CHT who subsequently developed superimposed pre-eclampsia had significantly lower PlGF concentrations adjusted for gestation at sampling than women with CKD and/or CHT without superimposed pre-eclampsia ($p<0.001$). There were no differences in PlGF concentrations and Z scores between women with CKD and/or CHT with superimposed pre-eclampsia and those with pre-eclampsia without pre-existing disease.

PlGF as predictive marker

Specificities and negative predictive values of PlGF<5th centile for superimposed pre-eclampsia (prior to onset) were high (>80%); sensitivity was maximal at 25^{+0}-28^{+6} weeks (66.7%, 95% CI 22.3-95.7%) (Supplementary Table 5).

PlGF as diagnostic marker for superimposed pre-eclampsia requiring delivery

Sensitivity and specificity for PlGF for diagnosis of superimposed pre-eclampsia requiring delivery within 14 days are shown in Table 5, and a threshold of PlGF <5th centile had the best diagnostic performance. Lower values of plasma PlGF were associated with superimposed pre-eclampsia requiring delivery within 14 days when assessed between 20^{+0}-36^{+6} weeks' gestation (ROC 0.85, SE 0.06) or between 20^{+0}-40^{+6} weeks' gestation (ROC 0.83, SE 0.08). Subgroup analyses did not reveal any differences in ROCs between groups at 20^{+0}-36^{+6} weeks: CKD alone 0.90 (SE 0.05); CHT alone 0.76 (SE 0.17); CKD with secondary CHT 0.77 (SE 0.17), nor at 20^{+0}-40^{+6} weeks: CKD alone 0.93 (SE 0.05); CHT alone 0.80 (SE 0.09); CKD with secondary CHT 0.78 (0.17). Clinical information relating to

women with false positive and false negative PlGF concentrations (<5th Centile) at 20^{+0}-36^{+6} weeks are shown in Supplementary Table 6. Only three women with low PlGF concentrations (1.8%) had uncomplicated outcomes. Of the 14 false positive results, three samples were taken before 22 weeks' gestation with no clinical suspicion of superimposed pre-eclampsia and eight women had clinical features of superimposed pre-eclampsia but did not meet study criteria for diagnosis and three women had uncomplicated deliveries. There were three false negative results including two in whom clinical suspicion was low, yet increments in blood pressure and proteinuria met study criteria for diagnosis and one woman with catastrophic antiphospholipid syndrome.

There was no significant difference in test performance at 20-40^{+6} weeks' for diagnosis of requirement for delivery for SPE within 14 days between white women (ROC 0.79 (SE 0.09) (n= 7 cases out of 68 women) and black women (ROC 0.90 (SE 0.05) n=7 out of 54 women). Comparison of PlGF test performance for prediction of preterm delivery <34 weeks' (ROC 0.66 (SE 0.02)), <37 weeks' (0.59 (0.07) and SGA <3rd centile (0.71 (0.06) were lower than for prediction of requirement for delivery within 14 days for superimposed pre-eclampsia (ROC 0.83 (SE 0.08)). Further analysis of the validation cohort was therefore not performed.

BNP, NGAL and relaxin

BNP concentrations were higher in women with pre-eclampsia (p=0.04) and superimposed pre-eclampsia (p<0.0001) than healthy controls, but was not discriminatory in women with CKD and/or CHT for superimposed pre-eclampsia . NGAL concentrations were higher in women with CKD and/or CHT with and without superimposed pre-eclampsia than healthy controls (p<0.0001) and was also non-discriminatory for superimposed pre-eclampsia . Relaxin was undetectable in three women with ovum donation pregnancies, who were excluded from further analysis. Women with CKD and/or CHT without superimposed pre-eclampsia had higher relaxin concentrations than healthy controls (P=0.014) but there were no differences between women with CKD and/or CHT with and without superimposed pre-eclampsia.

There was no relationship between PlGF, BNP, NGAL or serum relaxin and development of pregnancy associated sub-acute kidney injury, or deterioration in renal function at six weeks or six months postpartum.

Associations between markers, and with creatinine

There was no relationship between the PlGF and creatinine concentration, whereas creatinine and NGAL (R=0.75; P<0.0001) and BNP (R=0.37; P<0.0001) and relaxin (R=0.26; P<0.0001) concentrations

were significantly correlated. There was no association between the PlGF concentration and any of the other markers. There was no relationship between PlGF centiles and ethnicity.

Uterine and umbilical artery Doppler studies

Diagnostic performance for uterine and umbilical artery Doppler as predictors of the development of superimposed pre-eclampsia was poor (Supplementary Table 7).

Validation Cohort

PlGF, the best performing marker for determination of women with superimposed pre-eclampsia requiring delivery within 14 days, was validated in plasma samples from a second cohort of women with CKD and/or CHT, and compared to women without pre-existing disease (Figure 1b). Diagnostic performance of sFlt-1 was additionally evaluated in these women. Baseline demographics and maternal and neonatal outcomes are shown in Supplementary Tables 8 and 9.

PlGF, sFlt-1 and sFlt-1:PlGF as diagnostic markers

In this second cohort, women with superimposed pre-eclampsia requiring delivery within 14 days had lower PlGF concentrations and higher sFlt-1 and sFlt-1:PlGF ratios than women with CKD and/or CHT without superimposed pre-eclampsia($P<0.0001$); similarly significant differences in biomarker concentrations were seen in women with pre-eclampsia compared with women with no pre-existing disease($P<0.0001$). PlGF and sFlt-1 concentrations were not different between women with CKD and/or CHT and those without pre-existing disease who did not develop superimposed pre-eclampsia or pre-eclampsia, nor was there any difference in concentrations between women with pre-eclampsia and superimposed pre-eclampsia (Supplementary Table 10). The diagnostic utility of low PlGF concentrations in women with CKD and/or CHT for superimposed pre-eclampsia requiring delivery within 14 days for samples taken between 20^{+0}-36^{+6} weeks was confirmed (ROC 0.82; SE 0.06) in the validation cohort (Table 6). Diagnostic performance of sFlt-1 was also high (ROC 0.79; SE 0.06) but provided no significant incremental value when evaluated as the sFlt-1/PlGF ratio (0.83; SE 0.06). There were no differences in diagnostic performance of PlGF concentrations, sFlt-1 and sFlt-1:PlGF ratios between women with CKD and/or CHT and women without pre-existing disease (Supplementary Table 11). Sensitivity and specificity analysis of sFlt1-1: PLGF ratio>85 were comparable to PlGF <5th centile (Supplementary Table 12).

Discussion

The results of this study demonstrate that PlGF concentration has a good diagnostic performance for superimposed pre-eclampsia requiring delivery within 14 days in women with CKD and/or CHT (PlGF (<5th centile) which is comparable to PlGF test performance for pre-eclampsia in women with no underlying disease. This observation, being confirmed in a second cohort, provides promising evidence for potential use in the clinical setting but requires further validation. Identification of superimposed pre-eclampsia in women with CKD and/or CHT is substantially more challenging than detecting pre-eclampsia, as worsening hypertension and proteinuria, the conventional diagnostic parameters, are difficult to interpret when already present in early pregnancy. Furthermore, only 15 out of 173 women (8.7%) with superimposed pre-eclampsia had additional abnormal laboratory parameters other than serum creatinine. Over a quarter of all women with CKD and/or CHT required antenatal admission without delivery to investigate for superimposed pre-eclampsia thus confirming the need for an accurate test. Quantification of PlGF concentrations at time of suspected superimposed pre-eclampsia in women with CKD and/or CHT may be a useful adjunctive tool to aid diagnostic uncertainty.

To our knowledge, this study includes the largest reported number of women with CKD and/or CHT in whom longitudinal and diagnostic analyses of potential biomarkers for superimposed pre-eclampsia have been assessed. Direct comparison of markers with healthy controls and women with pre-eclampsia without pre-existing conditions enabled assessment of the influence of pre-existing disease. We found that PlGF and sFlt-1 concentrations in women with CKD and/or CHT follow the same gestational trajectories as women without pre-existing disease,[16, 17] supporting a substantial placental contribution to the development of superimposed pre-eclampsia whereas the association between maternal cardiac or renal function quantified by BNP, NGAL and relaxin and the onset of superimposed pre-eclampsia was not of diagnostic potential.

Diagnostic criteria for superimposed pre-eclampsia as written both in guidelines and reported in pregnancy studies in CKD and/or CHT are ambiguous.[18, 19] A strength of our study was the use of robust thresholds for diagnosis. Changes in blood pressure and proteinuria in normal and CKD pregnancies are well recognised, and therefore the use of arbitrary thresholds of hypertension and proteinuria is recognised as potentially problematic, and the thresholds chosen are likely to be imperfect, particularly as some women may have been delivered prior to the development of parameters of sufficient severity to reach criteria. However, in the absence of gestational reference ranges that accurately reflect anticipated fold changes in blood pressure and proteinuria for women with CKD and/or CHT this method was chosen for confirmation of superimposed pre-eclampsia, as reflected in the international definitions. Only three of 161 women with CKD and/or CHT with false

positive results had clinical courses which were uncomplicated. Importantly, the findings of our study suggest that PlGF concentrations might be used to define superimposed pre-eclampsia in the future, thus reducing the need for interpretation of ambiguous clinical parameters.

PlGF in the urine of pregnant women mirrors the plasma concentration[20] but accumulation of plasma PlGF in women with reduced renal function could confound interpretation of plasma PlGF. However, the absence of a correlation between PlGF and serum creatinine renders this unlikely, although as the number of women with severe CKD was few, this requires further assessment.

Two studies which included small numbers of women with chronic glomerulonephritis (n=15 and n=35) have suggested previously that PlGF and sFlt-1 may be useful markers for superimposed pre-eclampsia[21, 22] but a strength of the present, much larger study, is the demonstration of usefulness of PlGF across a spectrum of CKD aetiologies. Others report that PlGF and sFlt-1 concentrations discriminated between 34 women with pre-eclampsia (without pre-existing risk factors) and 23 women with CKD without superimposed pre-eclampsia but, unlike the present study women with superimposed pre-eclampsia were not investigated.[23] Longitudinal studies, confined to women with CHT, also report low PlGF and high sFlt-1 concentrations prior to onset and time of diagnosis of superimposed pre-eclampsia[24,25, 26] but findings have not been validated in a second cohort as reported here. Only one study of 313 women with CHT found no differences in PlGF in women with and without superimposed pre-eclampsia,[27] despite the cohort having a similar demographic profile and timing of delivery as the present study; the reasons for these discrepant results are unclear.

In our study one in five (20.4%) women with CKD without superimposed pre-eclampsia was delivered for other renal related indications including the presence of stable CKD. If confirmed by further validation, high PlGF concentrations may reduce clinician anxiety and therefore the incidence of unnecessary admission and preterm iatrogenic delivery due to uncertainty over the clinical significance of gestational change. However, a limitation of this study is the number of women with advanced disease and further study of PlGF in this population is needed. Markers of cardiac and renal function were not predictive or diagnostic of superimposed pre-eclampsia. Further haemodynamic assessments in women with CKD and/or CHT are needed to determine the contribution of pre-existing cardiac and vascular disease to the development of superimposed pre-eclampsia. In contrast with another report of high specificity of Doppler studies in pregnant women with CKD,[28] predictive performance of artery Doppler for prediction of superimposed pre-eclampsia was poor but may have been limited by non-standardised methods of measurements, frequency and number of scans performed.

Conclusions

The findings of this study support the role of PlGF as a diagnostic test for superimposed pre-eclampsia requiring delivery within 14 days for women with CKD and/or CHT, and suggest that impaired placental function rather than pre-existing maternal disease alone is a major contributor to the development of superimposed pre-eclampsia. Further investigation of the influence of pre-existing maternal disease on the process of early placentation is required.

Methods

Longitudinal cohort

Women were prospectively enrolled from two London Academic Health Science Centres (Imperial College and King's Health Partners) between June 2009 and September 2013. Ethical approval was provided by the National Research Ethics Service (NRES) (11/LO/1776) and the study was performed in accordance with the guidelines of the Declaration of Helsinki. Demographic information was recorded following written informed consent. Venous blood samples were taken up to four times during pregnancy and serum and plasma stored at -80°C. Maternal and perinatal outcome data were obtained by case note review after delivery. Definitions for study entry, and outcomes are based on International Society of Study of Hypertension in Pregnancy guidelines[29] (Supplementary Table 1). The following women were recruited i) women with CKD and/or CHT who did and did not develop superimposed pre-eclampsia ii) healthy controls iii) women with pre-eclampsia at time of disease (without pre-existing disease) iv) women with superimposed pre-eclampsia at time of disease, if they had not already been recruited longitudinally.

The most recent serum creatinine prior to pregnancy was recorded, and for women with no measurement available an approximation of eGFR before pregnancy was made by increasing the first recorded antenatal creatinine by 25% and calculation of eGFR by Modified Diet in Renal Disease calculation[30] an approach previously used by others.[3] Uterine and umbilical artery Doppler studies were recorded for women with CKD and/or CKD, and the predictive value for development of superimposed pre-eclampsia of mean uterine artery Doppler pressure indices >1.4 and bilateral notches at 20-24^{+6} weeks' and umbilical artery indices >95th centile at >28 weeks' gestation and within 14 days of delivery were analysed. All data and final diagnoses were entered by one researcher, confirmed by a second reviewer, and for complex cases, the diagnosis was adjudicated by a third senior reviewer, all without access to study (biomarker) results.

Acute kidney injury was defined as a 50% increase in serum creatinine within one week.[31] Pregnancy associated sub-acute kidney injury was defined as a 50% increase in serum creatinine from the lowest recorded value. Birth-weight was assessed by customised birthweight percentile (gestation related Optimal Weight),[32] and small for gestational age (SGA) reported as <3rd and <5th centile.

Validation cohort

Women requiring assessment for suspected pre-eclampsia were enrolled in a longitudinal cohort study approved by NRES (Ref 10/H0701/117), between January 2011 and February 2012 from seven consultant-led units in the UK and Ireland, and women with CKD and/or CHT or no pre-existing disease selected. Details have been described previously.[15] All samples were taken at time of

suspected disease in order to assess the diagnostic performance of the test at time of presentation and were categorised according to outcome at delivery.

Assay analyses

Plasma samples were tested without awareness of clinical outcomes, using the Triage PlGF Tests and CardioRenal (BNP, NGAL) Test (Alere, San Diego CA) according to the manufacturer's instructions. The assays use fluorescently labelled recombinant murine monoclonal antibodies (PlGF, BNP) or recombinant monoclonal antibodies (NGAL), detecting the biomarkers specifically and quantitatively within certain ranges (PlGF 12-3000 pg/ml; BNP 5-5000 pg/ml; NGAL 15-1300 pg/ml). The total precision (coefficient of variation) on plasma controls for PlGF at concentrations of 85 and 1300 pg/ml is 12.8% and 13.2% respectively; for BNP at concentrations of 78 and 3450 pg/ml is 9.2% and 13.9% respectively; for NGAL at concentrations of 98 and 1280 ng/ml is 12.5% and 13.5% respectively based on the manufacturer's package inserts generated before the study.

Serum relaxin was quantified using a specific enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (R&D Systems, Inc., Minneapolis, MN, USA), with an assay range of 7.81-500 pg/ml, sensitivity of 4.57 pg/ml and inter and intra-assay coefficients of variation of 3.8% and 4.3% respectively.

sflt-1 was quantified with a Luminex sandwich assay (Alere, San Diego CA), using a mouse-derived recombinant Fab conjugated to a magnetic bead as the capture, and a biotin-conjugated recombinant Fab as the assay detection, optimised for use in Luminex xMap technology (Alere, San Diego). The assay range was 0.006-27.87 pg/ml and coefficient of variation 10%.

Statistical analysis

Normality of distribution was explored using a Q-Q plot, and logarithmic transformations used where appropriate. Demographic data are presented as medians (interquartile range) (as not normally distributed) or frequencies (percentages). Mann-Whitney, and Fisher's exact test were used to test differences between groups. T-tests were used to test differences between biomarkers following logarithmic transformation to a normal distribution. PlGF concentrations greater than 19 weeks were transformed into a PlGF centile [33] and z-scores calculated. To avoid multiple testing, data were corrected for the inclusion of women who had provided several samples throughout the same pregnancy, using interval regression analysis with random-effect modelling for individual clustering.[34] Interval regression also allows samples measured as below (above) the lower (upper) limit of detection to be treated as being in an appropriate range, rather than replacing them with a single number. The last visit for each woman of the longitudinal cohort taken after 20 weeks'

gestation was included for analysis of diagnostic performance of the primary outcome (superimposed pre-eclampsia requiring delivery within 14 days). Test performance was evaluated as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and receiver operator characteristic (ROC) in both cohorts for PlGF <5th Centile and sFlt-1:PlGF ratio >85 as described by others. [12,31]Spearman's rank correlations were performed on the last sample taken from each woman in order to prevent confounding by frequent sampling within an individual. Statistical analysis was performed in the statistical package Stata (Version 13) and IBM Statistical Package for the Social Sciences (SPSS) (Version 21). The study is reported in accordance with STAndards for the Reporting of Diagnostic accuracy studies (STARD) guidelines.

Sample size calculation

With 29 cases and 94 controls in the validation set, an unbiased estimate can be obtained within 15% of the true sensitivity and within 8% of the true specificity using 95% confidence intervals around values of 80% sensitivity and specificity.

Role of the funding source

This was an investigator-led study; the funder had no role in study design, patient recruitment, data collection, analysis, interpretation, nor in writing of the manuscript or decision to submit for publication.

Disclosure

KB, PTS, LL, CNP, CG, PW, LP and LC report no conflicts.

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Titles and Legends

Figure 1a: Longitudinal Cohort – Flow Diagram of participants

Figure 1b: Validation Cohort – Flow Diagram of participants

Figure 2: Placental growth factor concentrations in healthy controls, women with CHT and/or CKD with and without superimposed pre-eclampsia in a longitudinal cohort and in women with time of disease pre-eclampsia according to gestation in weeks

Diagnostic and predictive biomarkers for superimposed pre-eclampsia

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Abstract

Women with chronic kidney disease (CKD) and chronic hypertension (CHT) frequently develop superimposed pre-eclampsia but distinction from pre-existing disease is challenging. Plasma placental growth factor (PlGF), B-type natriuretic peptide (BNP), neutrophil gelatinase-associated lipocalin (NGAL) and serum relaxin concentrations were quantified in a longitudinal prospective cohort of women with CKD (n=121), CHT (n=44) and healthy controls (n=79) and biomarker concentrations compared with women with pre-eclampsia without pre-existing disease (n=32). Test performance was evaluated for diagnosis of superimposed pre-eclampsia requiring delivery within 14 days of sampling. PlGF was evaluated as a promising marker in a validation cohort in a validation cohort of women with suspected pre-eclampsia (CKD n=29; CHT n=94; superimposed pre-eclampsia requiring delivery within 14 days n=29) and compared to women without pre-existing disease (No pre-eclampsia n=290; pre-eclampsia requiring delivery within 14 days n= 176). Between 20^{+0}-36^{+6} weeks' gestation, PlGF had high diagnostic accuracy for superimposed pre-eclampsia requiring delivery within 14 days (receiver operator characteristic [ROC] 0.85; SE 0.06) and was confirmed (ROC 0.82; SE 0.06) in the validation cohort. Plasma NGAL, BNP and serum relaxin concentrations were not discriminatory for superimposed pre-eclampsia. A low maternal plasma PlGF concentration could be a useful adjunct to guide decisions regarding admission and delivery for superimposed pre-eclampsia.

Key Words: Chronic kidney disease; Endothelium; Proteinuria

Introduction

Pre-eclampsia is estimated to complicate 2-8% of all pregnancies;[1] however superimposed pre-eclampsia is reported to affect approximately 26% of pregnant women with chronic hypertension (CHT)[2] and 22-75% of women with chronic kidney disease (CKD).[3] The diagnosis of pre-eclampsia when superimposed upon chronic kidney disease (CKD) and chronic hypertension (CHT) is challenging as it may be clinically indistinguishable from benign gestational progression of pre-existing hypertension and proteinuria, which often coexist.[4] Superimposed pre-eclampsia is frequently associated with poor maternal and fetal outcomes. Therefore early and accurate diagnosis is essential to allow timely intervention, whilst misdiagnosis may also lead to unnecessary admissions and iatrogenic preterm delivery.

Current hypotheses describe abnormal placental perfusion and predisposing maternal factors[5] (including cardiac, vascular and renal dysfunction) in the genesis of pre-eclampsia, but the relevant contribution of placental and maternal influences to development of superimposed pre-eclampsia is poorly understood. The aims of this study were to evaluate the predictive and diagnostic performance for superimposed pre-eclampsia of markers of placental, cardiac and renal function which have previously been implicated in pre-eclampsia in women without underlying disease.

The markers studied were placental growth factor (PlGF), soluble fms-like tyrosine kinase receptor (sFlt-1), B type natriuretic peptide (BNP), neutrophil gelatinase associated lipocalin (NGAL), and relaxin. PlGF, an angiogenic protein synthesised by syncytiotrophoblasts increases in the blood of healthy pregnant women until 26-30 weeks and then fall towards term[6]; low plasma PlGF has been reported in women with pre-eclampsia.[7] Conversely sFlt-1, an anti-angiogenic protein which binds to PlGF preventing interaction with endothelial receptors is raised in women with pre-eclampsia.[8] Higher than normal concentrations of BNP[9, 10] are observed in women with pre-eclampsia; BNP is released with cardiac ventricular strain, and is raised prior to diagnosis in women with early onset pre-eclampsia.[11] NGAL , an early marker of acute kidney injury (AKI) has been associated with pre-eclampsia in women without pre-existing disease[12,13] and relaxin is an ovarian hormone known to play an important physiological role in the renal adaptation to pregnancy.[14]

Measurement of these biomarkers was undertaken in four groups of women with CKD and/or CHT with superimposed pre-eclampsia; CKD and/or CHT without superimposed pre-eclampsia; pre-eclampsia without CKD and/or CHT and healthy controls. We validated the best performing marker in a second cohort of women with suspected pre-eclampsia or superimposed pre-eclampsia. The primary outcome of superimposed pre-eclampsia requiring delivery within 14 days was chosen to provide a clinically relevant endpoint that reflects current management strategies.[15]

Results

Longitudinal Cohort

288 women were recruited to the study (Figure 1a). Of those consented, 11 were lost to follow-up and one was subsequently recruited to a treatment trial for pre-eclampsia. The remaining 276 women provided 471 samples for analysis.

Demographics

Baseline demographic data are presented in Table 1. Details of underlying disease in women with CKD and/ or CHT are shown in Supplementary Table 2. There were no demographic differences between women with CKD and/or CHT who did or did not develop superimposed pre-eclampsia.

Demographics for women with CKD and/or CHT according to severity of CKD are shown in Supplementary Table 3. Twenty-five (21.6%) of 121 women with CKD had the diagnosis of CKD made during the index pregnancy, including six (26%) of the 23 women with CKD who subsequently developed superimposed pre-eclampsia.

Maternal outcomes

Maternal outcomes are shown in Table 2. Forty women (24.2%) were diagnosed with superimposed pre-eclampsia. Twenty-three women with CKD (19.0%) developed superimposed pre-eclampsia, including 8/61 women (13.1%) without secondary CHT, and 15/45 (37.5%) with CHT (P=0.1)), whereas 12/39 women (30.8%) with primary hypertension developed superimposed pre-eclampsia (excluding 5 women who were recruited at time of disease). Only eight women with superimposed pre-eclampsia (20%) had elevated alanine transaminase (ALT) or low platelets.

Women with superimposed pre-eclampsia were delivered for indications related to its development including maternal hypertension (n=15; 37.5%), deteriorating renal function (6; 15%), liver abnormalities (2; 5%), neurological symptoms (2; 5%), fetal indications (10; 25%) and post 37 weeks' gestation (2; 5%) and the indication was not clear in two cases (2; 5%). Forty-four (45%) of 98 women with CKD without superimposed pre-eclampsia had iatrogenic delivery for reasons including 'deteriorating renal function' (12; 27%), increasing proteinuria (2; 4.5%), increasing hypertension (2; 4.5%) and the presence of stable CKD (4; 9.0%). Women with CKD and/or CHT had more antenatal admissions than healthy controls (P<0.0001). Women with superimposed pre-eclampsia were also more likely to have longer peripartum admissions than women with those without (p<0.0001), but did not have any more antenatal admissions.

Acute kidney injury

Overall, 29 (24.2%) women with CKD and 10 (22.7%) women with CHT developed pregnancy associated sub-acute kidney injury. Women with CKD who developed acute kidney injury, pregnancy associated sub-acute kidney injury or postpartum progression of renal disease including those who also met study criteria for superimposed pre-eclampsia are described in Table 3. None of the women required renal replacement therapy either during or after pregnancy, except one woman already receiving haemodialysis, and there were no maternal deaths.

Neonatal outcomes

Neonatal outcomes are shown in Table 4. Neonatal outcomes according to underlying presence of CHT and CKD stage are shown in Supplementary Table 4.

PlGF changes with gestation

Figure 2 shows the gestational profile of PlGF concentrations in 471 samples from 276 women. Following interval regression (46 left-censored observations, one right-censored) adjusted for gestation at sampling, women with pre-eclampsia without pre-existing disease had lower PlGF concentrations ($p<0.001$) and Z- scores than healthy controls ($p<0.001$) at all gestational time points. Women with CKD and/or CHT who subsequently developed superimposed pre-eclampsia had significantly lower PlGF concentrations adjusted for gestation at sampling than women with CKD and/or CHT without superimposed pre-eclampsia ($p<0.001$). There were no differences in PlGF concentrations and Z scores between women with CKD and/or CHT with superimposed pre-eclampsia and those with pre-eclampsia without pre-existing disease.

PlGF as predictive marker

Specificities and negative predictive values of PlGF<5th centile for superimposed pre-eclampsia (prior to onset) were high (>80%); sensitivity was maximal at 25^{+0}-28^{+6} weeks (66.7%, 95% CI 22.3-95.7%) (Supplementary Table 5).

PlGF as diagnostic marker for superimposed pre-eclampsia requiring delivery

Sensitivity and specificity for PlGF for diagnosis of superimposed pre-eclampsia requiring delivery within 14 days are shown in Table 5, and a threshold of PlGF <5th centile had the best diagnostic performance. Lower values of plasma PlGF were associated with superimposed pre-eclampsia requiring delivery within 14 days when assessed between 20^{+0}-36^{+6} weeks' gestation (ROC 0.85, SE 0.06) or between 20^{+0}-40^{+6} weeks' gestation (ROC 0.83, SE 0.08). Subgroup analyses did not reveal any differences in ROCs between groups at 20^{+0}-36^{+6} weeks: CKD alone 0.90 (SE 0.05); CHT alone 0.76 (SE 0.17); CKD with secondary CHT 0.77 (SE 0.17), nor at 20^{+0}-40^{+6} weeks: CKD alone 0.93 (SE 0.05); CHT alone 0.80 (SE 0.09); CKD with secondary CHT 0.78 (0.17). Clinical information relating to

women with false positive and false negative PlGF concentrations (<5th Centile) at 20^{+0}-36^{+6} weeks are shown in Supplementary Table 6. Only three women with low PlGF concentrations (1.8%) had uncomplicated outcomes. Of the 14 false positive results, three samples were taken before 22 weeks' gestation with no clinical suspicion of superimposed pre-eclampsia and eight women had clinical features of superimposed pre-eclampsia but did not meet study criteria for diagnosis and three women had uncomplicated deliveries. There were three false negative results including two in whom clinical suspicion was low, yet increments in blood pressure and proteinuria met study criteria for diagnosis and one woman with catastrophic antiphospholipid syndrome.

There was no significant difference in test performance at 20-40^{+6} weeks' for diagnosis of requirement for delivery for SPE within 14 days between white women (ROC 0.79 (SE 0.09) (n= 7 cases out of 68 women) and black women (ROC 0.90 (SE 0.05) n=7 out of 54 women). Comparison of PlGF test performance for prediction of preterm delivery <34 weeks' (ROC 0.66 (SE 0.02)), <37 weeks' (0.59 (0.07) and SGA <3rd centile (0.71 (0.06) were lower than for prediction of requirement for delivery within 14 days for superimposed pre-eclampsia (ROC 0.83 (SE 0.08)). Further analysis of the validation cohort was therefore not performed.

BNP, NGAL and relaxin

BNP concentrations were higher in women with pre-eclampsia (p=0.04) and superimposed pre-eclampsia (p<0.0001) than healthy controls, but was not discriminatory in women with CKD and/or CHT for superimposed pre-eclampsia . NGAL concentrations were higher in women with CKD and/or CHT with and without superimposed pre-eclampsia than healthy controls (p<0.0001) and was also non-discriminatory for superimposed pre-eclampsia . Relaxin was undetectable in three women with ovum donation pregnancies, who were excluded from further analysis. Women with CKD and/or CHT without superimposed pre-eclampsia had higher relaxin concentrations than healthy controls (P=0.014) but there were no differences between women with CKD and/or CHT with and without superimposed pre-eclampsia.

There was no relationship between PlGF, BNP, NGAL or serum relaxin and development of pregnancy associated sub-acute kidney injury, or deterioration in renal function at six weeks or six months postpartum.

Associations between markers, and with creatinine

There was no relationship between the PlGF and creatinine concentration, whereas creatinine and NGAL (R=0.75; P<0.0001) and BNP (R=0.37; P<0.0001) and relaxin (R=0.26; P<0.0001) concentrations

were significantly correlated. There was no association between the PlGF concentration and any of the other markers. There was no relationship between PlGF centiles and ethnicity.

Uterine and umbilical artery Doppler studies

Diagnostic performance for uterine and umbilical artery Doppler as predictors of the development of superimposed pre-eclampsia was poor (Supplementary Table 7).

Validation Cohort

PlGF, the best performing marker for determination of women with superimposed pre-eclampsia requiring delivery within 14 days, was validated in plasma samples from a second cohort of women with CKD and/or CHT, and compared to women without pre-existing disease (Figure 1b). Diagnostic performance of sFlt-1 was additionally evaluated in these women. Baseline demographics and maternal and neonatal outcomes are shown in Supplementary Tables 8 and 9.

PlGF, sFlt-1 and sFlt-1:PlGF as diagnostic markers

In this second cohort, women with superimposed pre-eclampsia requiring delivery within 14 days had lower PlGF concentrations and higher sFlt-1 and sFlt-1:PlGF ratios than women with CKD and/or CHT without superimposed pre-eclampsia($P<0.0001$); similarly significant differences in biomarker concentrations were seen in women with pre-eclampsia compared with women with no pre-existing disease($P<0.0001$). PlGF and sFlt-1 concentrations were not different between women with CKD and/or CHT and those without pre-existing disease who did not develop superimposed pre-eclampsia or pre-eclampsia, nor was there any difference in concentrations between women with pre-eclampsia and superimposed pre-eclampsia (Supplementary Table 10). The diagnostic utility of low PlGF concentrations in women with CKD and/or CHT for superimposed pre-eclampsia requiring delivery within 14 days for samples taken between 20^{+0}-36^{+6} weeks was confirmed (ROC 0.82; SE 0.06) in the validation cohort (Table 6). Diagnostic performance of sFlt-1 was also high (ROC 0.79; SE 0.06) but provided no significant incremental value when evaluated as the sFlt-1/PlGF ratio (0.83; SE 0.06). There were no differences in diagnostic performance of PlGF concentrations, sFlt-1 and sFlt-1:PlGF ratios between women with CKD and/or CHT and women without pre-existing disease (Supplementary Table 11). Sensitivity and specificity analysis of sFlt1-1: PLGF ratio>85 were comparable to PlGF <5th centile (Supplementary Table 12).

Discussion

The results of this study demonstrate that PlGF concentration has a good diagnostic performance for superimposed pre-eclampsia requiring delivery within 14 days in women with CKD and/or CHT (PlGF (<5th centile) which is comparable to PlGF test performance for pre-eclampsia in women with no underlying disease. This observation, being confirmed in a second cohort, provides promising evidence for potential use in the clinical setting but requires further validation. Identification of superimposed pre-eclampsia in women with CKD and/or CHT is substantially more challenging than detecting pre-eclampsia, as worsening hypertension and proteinuria, the conventional diagnostic parameters, are difficult to interpret when already present in early pregnancy. Furthermore, only 15 out of 173 women (8.7%) with superimposed pre-eclampsia had additional abnormal laboratory parameters other than serum creatinine. Over a quarter of all women with CKD and/or CHT required antenatal admission without delivery to investigate for superimposed pre-eclampsia thus confirming the need for an accurate test. Quantification of PlGF concentrations at time of suspected superimposed pre-eclampsia in women with CKD and/or CHT may be a useful adjunctive tool to aid diagnostic uncertainty.

To our knowledge, this study includes the largest reported number of women with CKD and/or CHT in whom longitudinal and diagnostic analyses of potential biomarkers for superimposed pre-eclampsia have been assessed. Direct comparison of markers with healthy controls and women with pre-eclampsia without pre-existing conditions enabled assessment of the influence of pre-existing disease. We found that PlGF and sFlt-1 concentrations in women with CKD and/or CHT follow the same gestational trajectories as women without pre-existing disease,[16, 17] supporting a substantial placental contribution to the development of superimposed pre-eclampsia whereas the association between maternal cardiac or renal function quantified by BNP, NGAL and relaxin and the onset of superimposed pre-eclampsia was not of diagnostic potential.

Diagnostic criteria for superimposed pre-eclampsia as written both in guidelines and reported in pregnancy studies in CKD and/or CHT are ambiguous.[18, 19] A strength of our study was the use of robust thresholds for diagnosis. Changes in blood pressure and proteinuria in normal and CKD pregnancies are well recognised, and therefore the use of arbitrary thresholds of hypertension and proteinuria is recognised as potentially problematic, and the thresholds chosen are likely to be imperfect, particularly as some women may have been delivered prior to the development of parameters of sufficient severity to reach criteria. However, in the absence of gestational reference ranges that accurately reflect anticipated fold changes in blood pressure and proteinuria for women with CKD and/or CHT this method was chosen for confirmation of superimposed pre-eclampsia, as reflected in the international definitions. Only three of 161 women with CKD and/or CHT with false

positive results had clinical courses which were uncomplicated. Importantly, the findings of our study suggest that PlGF concentrations might be used to define superimposed pre-eclampsia in the future, thus reducing the need for interpretation of ambiguous clinical parameters.

PlGF in the urine of pregnant women mirrors the plasma concentration[20] but accumulation of plasma PlGF in women with reduced renal function could confound interpretation of plasma PlGF. However, the absence of a correlation between PlGF and serum creatinine renders this unlikely, although as the number of women with severe CKD was few, this requires further assessment.

Two studies which included small numbers of women with chronic glomerulonephritis (n=15 and n=35) have suggested previously that PlGF and sFlt-1 may be useful markers for superimposed pre-eclampsia[21, 22] but a strength of the present, much larger study, is the demonstration of usefulness of PlGF across a spectrum of CKD aetiologies. Others report that PlGF and sFlt-1 concentrations discriminated between 34 women with pre-eclampsia (without pre-existing risk factors) and 23 women with CKD without superimposed pre-eclampsia but, unlike the present study women with superimposed pre-eclampsia were not investigated.[23] Longitudinal studies, confined to women with CHT, also report low PlGF and high sFlt-1 concentrations prior to onset and time of diagnosis of superimposed pre-eclampsia[24,25, 26] but findings have not been validated in a second cohort as reported here. Only one study of 313 women with CHT found no differences in PlGF in women with and without superimposed pre-eclampsia,[27] despite the cohort having a similar demographic profile and timing of delivery as the present study; the reasons for these discrepant results are unclear.

In our study one in five (20.4%) women with CKD without superimposed pre-eclampsia was delivered for other renal related indications including the presence of stable CKD. If confirmed by further validation, high PlGF concentrations may reduce clinician anxiety and therefore the incidence of unnecessary admission and preterm iatrogenic delivery due to uncertainty over the clinical significance of gestational change. However, a limitation of this study is the number of women with advanced disease and further study of PlGF in this population is needed. Markers of cardiac and renal function were not predictive or diagnostic of superimposed pre-eclampsia. Further haemodynamic assessments in women with CKD and/or CHT are needed to determine the contribution of pre-existing cardiac and vascular disease to the development of superimposed pre-eclampsia. In contrast with another report of high specificity of Doppler studies in pregnant women with CKD,[28] predictive performance of artery Doppler for prediction of superimposed pre-eclampsia was poor but may have been limited by non-standardised methods of measurements, frequency and number of scans performed.

Conclusions

The findings of this study support the role of PlGF as a diagnostic test for superimposed pre-eclampsia requiring delivery within 14 days for women with CKD and/or CHT, and suggest that impaired placental function rather than pre-existing maternal disease alone is a major contributor to the development of superimposed pre-eclampsia. Further investigation of the influence of pre-existing maternal disease on the process of early placentation is required.

Methods

Longitudinal cohort

Women were prospectively enrolled from two London Academic Health Science Centres (Imperial College and King's Health Partners) between June 2009 and September 2013. Ethical approval was provided by the National Research Ethics Service (NRES) (11/LO/1776) and the study was performed in accordance with the guidelines of the Declaration of Helsinki. Demographic information was recorded following written informed consent. Venous blood samples were taken up to four times during pregnancy and serum and plasma stored at -80^{o}C. Maternal and perinatal outcome data were obtained by case note review after delivery. Definitions for study entry, and outcomes are based on International Society of Study of Hypertension in Pregnancy guidelines[29] (Supplementary Table 1). The following women were recruited i) women with CKD and/or CHT who did and did not develop superimposed pre-eclampsia ii) healthy controls iii) women with pre-eclampsia at time of disease (without pre-existing disease) iv) women with superimposed pre-eclampsia at time of disease, if they had not already been recruited longitudinally.

The most recent serum creatinine prior to pregnancy was recorded, and for women with no measurement available an approximation of eGFR before pregnancy was made by increasing the first recorded antenatal creatinine by 25% and calculation of eGFR by Modified Diet in Renal Disease calculation[30] an approach previously used by others.[3] Uterine and umbilical artery Doppler studies were recorded for women with CKD and/or CKD, and the predictive value for development of superimposed pre-eclampsia of mean uterine artery Doppler pressure indices >1.4 and bilateral notches at 20-24^{+6} weeks' and umbilical artery indices >95th centile at >28 weeks' gestation and within 14 days of delivery were analysed. All data and final diagnoses were entered by one researcher, confirmed by a second reviewer, and for complex cases, the diagnosis was adjudicated by a third senior reviewer, all without access to study (biomarker) results.

Acute kidney injury was defined as a 50% increase in serum creatinine within one week.[31] Pregnancy associated sub-acute kidney injury was defined as a 50% increase in serum creatinine from the lowest recorded value. Birth-weight was assessed by customised birthweight percentile (gestation related Optimal Weight),[32] and small for gestational age (SGA) reported as <3rd and <5th centile.

Validation cohort

Women requiring assessment for suspected pre-eclampsia were enrolled in a longitudinal cohort study approved by NRES (Ref 10/H0701/117), between January 2011 and February 2012 from seven consultant-led units in the UK and Ireland, and women with CKD and/or CHT or no pre-existing disease selected. Details have been described previously.[15] All samples were taken at time of

suspected disease in order to assess the diagnostic performance of the test at time of presentation and were categorised according to outcome at delivery.

Assay analyses

Plasma samples were tested without awareness of clinical outcomes, using the Triage PlGF Tests and CardioRenal (BNP, NGAL) Test (Alere, San Diego CA) according to the manufacturer's instructions. The assays use fluorescently labelled recombinant murine monoclonal antibodies (PlGF, BNP) or recombinant monoclonal antibodies (NGAL), detecting the biomarkers specifically and quantitatively within certain ranges (PlGF 12-3000 pg/ml; BNP 5-5000 pg/ml; NGAL 15-1300 pg/ml). The total precision (coefficient of variation) on plasma controls for PlGF at concentrations of 85 and 1300 pg/ml is 12.8% and 13.2% respectively; for BNP at concentrations of 78 and 3450 pg/ml is 9.2% and 13.9% respectively; for NGAL at concentrations of 98 and 1280 ng/ml is 12.5% and 13.5% respectively based on the manufacturer's package inserts generated before the study.

Serum relaxin was quantified using a specific enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (R&D Systems, Inc., Minneapolis, MN, USA), with an assay range of 7.81-500 pg/ml, sensitivity of 4.57 pg/ml and inter and intra-assay coefficients of variation of 3.8% and 4.3% respectively.

sflt-1 was quantified with a Luminex sandwich assay (Alere, San Diego CA), using a mouse-derived recombinant Fab conjugated to a magnetic bead as the capture, and a biotin-conjugated recombinant Fab as the assay detection, optimised for use in Luminex xMap technology (Alere, San Diego). The assay range was 0.006-27.87 pg/ml and coefficient of variation 10%.

Statistical analysis

Normality of distribution was explored using a Q-Q plot, and logarithmic transformations used where appropriate. Demographic data are presented as medians (interquartile range) (as not normally distributed) or frequencies (percentages). Mann-Whitney, and Fisher's exact test were used to test differences between groups. T-tests were used to test differences between biomarkers following logarithmic transformation to a normal distribution. PlGF concentrations greater than 19 weeks were transformed into a PlGF centile [33] and z-scores calculated. To avoid multiple testing, data were corrected for the inclusion of women who had provided several samples throughout the same pregnancy, using interval regression analysis with random-effect modelling for individual clustering.[34] Interval regression also allows samples measured as below (above) the lower (upper) limit of detection to be treated as being in an appropriate range, rather than replacing them with a single number. The last visit for each woman of the longitudinal cohort taken after 20 weeks'

gestation was included for analysis of diagnostic performance of the primary outcome (superimposed pre-eclampsia requiring delivery within 14 days). Test performance was evaluated as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and receiver operator characteristic (ROC) in both cohorts for PlGF <5th Centile and sFlt-1:PlGF ratio >85 as described by others. [12,31]Spearman's rank correlations were performed on the last sample taken from each woman in order to prevent confounding by frequent sampling within an individual. Statistical analysis was performed in the statistical package Stata (Version 13) and IBM Statistical Package for the Social Sciences (SPSS) (Version 21). The study is reported in accordance with STAndards for the Reporting of Diagnostic accuracy studies (STARD) guidelines.

Sample size calculation

With 29 cases and 94 controls in the validation set, an unbiased estimate can be obtained within 15% of the true sensitivity and within 8% of the true specificity using 95% confidence intervals around values of 80% sensitivity and specificity.

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This was an investigator-led study; the funder had no role in study design, patient recruitment, data collection, analysis, interpretation, nor in writing of the manuscript or decision to submit for publication.

Disclosure

KB, PTS, LL, CNP, CG, PW, LP and LC report no conflicts.

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Titles and Legends

Figure 1a: Longitudinal Cohort – Flow Diagram of participants

Figure 1b: Validation Cohort – Flow Diagram of participants

Figure 2: Placental growth factor concentrations in healthy controls, women with CHT and/or CKD with and without superimposed pre-eclampsia in a longitudinal cohort and in women with time of disease pre-eclampsia according to gestation in weeks

Table 1: Longitudinal cohort: Characteristics at First Antenatal Visit and Enrolment

Characteristic	Healthy controls (N=79)	Pre-eclampsia (N=32)	CKD and/or CHT without superimposed pre-eclampsia (N=125)	CKD and/or CHT with superimposed pre-eclampsia (N=40)
Age at booking (years)	32.0	29.0	33.0	33.5
Median (IQR)	(28.0, 36.0)	(26.0, 35.5)	(29.0, 37.0)	(28.2, 37.0)
BMI (kg/m^2)	22.4*	28.6*	26.3	27.1
Median (IQR)	(20.6, 24.9)	(24.2, 32.3)	(22.8, 31.5)	(23.8, 32.3)
Ethnicity	.			
White	54 (68.4%)	14 (43.8%)	58 (46.4%)	15 (37.5%)
Black	14 (17.7%)$^{\$}$	12 (37.5%)$^{\$}$	43 (34.4%)	20 (50.0%)
Asian	5 (6.3%)	2 (6.3%)	13 (10.4%)	2 (5.0%)
Other	6 (7.6%)	4 (12.5%)	11 (8.8%)	3 (7.5%)
Assisted conception	1 (1.2%)	2 (6.3%)	5 (4.0%)	2 (5.0%)
Nulliparous	49 (62.0%)	24 (75%)	63 (50.4%)	16 (40.0%)
Previous Fetal Loss				
<12 weeks (≥1)	17 (21.5%)	10 (31.3%)	27 (21.6%)$^{\#}$	16 (40%)$^{\#}$
12-24 weeks (≥1)	2 (2.5%)	2 (6.2%)	12 (9.6%)	4 (10.0%)
>24 weeks (≥1)	0	0	6 (4.8%)	6 (15%)
First antenatal SBP Median (IQR)	102 (100, 110)	112 (109, 123)*	120 (110, 130)$^{\#\#}$	124 (115, 137)$^{\#\#}$
First antenatal DBP Median (IQR)	63 (60, 70)	72 (66, 80)*	78 (70, 87)$^{\#\#}$	80 (71, 94)$^{\#\#}$
Proteinuria at booking	0	0	N=122 32 (26.2%)	N=40 7 (17.5%)
Smoking				
Never	73 (92.4%)	26 (81.3%)	115 (92.0%)	35 (87.5%)
Current smoker	2 (2.5%)	1 (3.1%)	2 (1.6%)	2 (5.0%)
Stopped pre/during pregnancy	4 (5.1%)	5 (15.6%)	8 (6.4%)	3 (7.5%)
Type 1 DM	0	1 (3.1%)	2 (1.6%) SPK	2 (5.0%) SPK
Type 2 DM	0	1 (3.1%)	2 (1.6%)	1 (2.5%)
Aspirin Use	1 (1.3%)*	7 (21.9%)*	105 (84.0%)	31 (77.5%)
Aspirin started before 12 weeks' gestation	1 (100%)	3 (50.0%)	72 (69.9%)	18 (62.1%)
Median (IQR) gestation of start of aspirin	-	14.5 (3.0, 27.5)	11 (4.0, 13.0)	12 (1.5, 14.5)

CKD: Chronic Kidney Disease; CHT: Chronic Hypertension; DM: Diabetes mellitus; SPK: Simultaneous pancreas kidney transplant

Ethnicity – all comparisons with white ethnicity

Healthy controls v pre-eclampsia - *P<0.001; **P=0.008; $^{\$}$P=0.012

CKD and/or CHT without superimposed pre-eclampsia v superimposed pre-eclampsia – $^{\#}$P=0.03; $^{\#\#}$P=0.044

Table 1: Longitudinal cohort: maternal outcomes in women with pre-eclampsia, healthy controls, chronic hypertension and/or chronic kidney disease with and without superimposed pre-eclampsia.

Outcome	Healthy controls (N=79)	Pre-eclampsia (N=32)	CKD and/or CHT without superimposed pre-eclampsia (N=125)	CKD and/or CHT with superimposed pre-eclampsia (N=40)
Onset of labour				
Spontaneous onset	72 (91.1%)	1 (3.1%)	46 (36.8%)	0
Induction of labour	4 (5.1%)*	27 (84.4%)*	52 (41.6%)*	23 (57.5%)*
No labour	3 (3.8%)*	4 (12.5%)*	27 (21.6%)*	17 (42.5%)*
Mode of delivery				
Unassisted vaginal	59 (74.7%)	5 (15.6%)	51 (40.8%)	3 (7.5%)
Elective Caesarean section	3 (3.8%)**	4 (12.5%)**	27 (21.6%)*	17 (42.5%)*
Emergency Caesarean section	5 (6.3%)*	19 (59.4%)*	27 (21.6%)*	19 (47.5%)*
Assisted vaginal	16 (20.5%)	4 (12.5%)	20 (16.0%)	1 (2.5%)
Details of preeclampsia				
Severe hypertension (SBP>160/ DBP>110 mmHg)	0	19 (61.3%)	15 (12.6%)*	34 (85.0%)*
Highest SBP Median (IQR)	-	165 (155, 178)	135 (125, 150) *	170 (160, 179) *
Highest DBP Median (IQR)	-	100 (93, 107)	90 (80, 98.8) *	103.5 (98.5, 113)*
Highest 24 hr urine collection protein (g/24hrs) Median (IQR)		0.9 (0.37, 2.01)	0.39 (0.28, 0.71)$$	0.79 (0.61, 1.55)$$
Highest pregnancy urine protein:creatinine ratio Median (IQR)		59 (29.8, 139)	60 (34, 180)#	79 (51.7, 113.7)#
Doubling of Proteinuria	0	-	42 (33.9%)*	27 (67.5%)*
Alanine aminotransferase ≥70 iu/L	-	1 (3.1%)	4 (3.2%)$	6 (15.4%)$
Platelet count <100 x10^9/l	-	1 (3.1%)	2 (1.6%)	2 (5.1%)
Required intravenous antihypertensive drugs	0	8 (25.0%)	0*	7 (17.5%)*
Required intravenous Magnesium Sulfate	0	4 (12.5%)	0*	10 (25.0%)*
Additional antihypertensive within 4 weeks of delivery	-	-	11 (9.2%)*	32 (82.1%)*
Admission details				
One or more other antenatal admission	3 (3.8%)***	5 (15.6%)***	37 (31.6%)	11 (27.5%)
Maternal peripartum	2 (1, 4)*	14 (9.7, 22)*	3 (2, 6) *	14 (6, 28) *

length of stay Median (IQR)				
SBP on discharge Median (IQR)	115 (106, 121)*	135 (127, 146)*	130 (120, 139)##	135 (125, 142)##
DBP on discharge Median (IQR)	71 (66, 77)*	85 (78, 91)*	83 (73, 88)	83 (78, 88)

Comparison a) between healthy control women and pre-eclampsia b) between women with CKD and/or CHT with and without superimposed pre-eclampsia

*P<0.0001; **P=0.0038; ***P=0.043; $P=0.014; $$ P=0.012; #P=0.002; ## P=0.028

Labour onset: Comparison made with spontaneous labour

Mode of delivery: Comparison made with vaginal delivery

SBP: Systolic Blood pressure; DBP: Diastolic Blood Pressure; IV: Intravenous

Table 1: Longitudinal cohort: Women with chronic kidney disease or chronic hypertension developing acute kidney injury and pregnancy associated sub-acute kidney injury and, and postpartum renal function

	CHT	CKD Stage 1	CKD Stage 2	CKD Stage 3	CKD Stage 4
	N=44	N=57	N=30	N=27	N=6
≥2+ Proteinuria at booking	0	21 (38.2%)	4 (12.9%)	11 (40.7%)	3 (50.0%)
Acute kidney injury	0	0	1 (3.3%)	1 (3.7%)	0
Pregnancy associated sub-acute kidney injury	10 (22.7%)	10 (17.5%)	8 (26.7%)	10 (37.0%)	1 (16.7%)
6 weeks postpartum	-	N=17	N=9	N=15	N=3
Median (IQR) decline in GFR mls/min/1.73m^2	-	12 (-7, 18)	7 (-0.5, 13)	6 (0, 9)	3 (0, 11)
≥25% reduction in GFR					
All women	-	2 (11.8%)	0	3 (20.0%)	1 (33.3%)
Women with SPE	-	0	0	1 (33.3%)	1 (100%)
6 months postpartum		N=26	N=13	N=17	N=3
Median (IQR) decline in GFR mls/min/1.73m^2	-	2 (-7.5, 22.5)	10 (-2.5, 13)	7 (0, 14.0)	6 (0, 13)
≥25% reduction in GFR					
All women	-	2 (7.7%)	2 (15.4%)	4 (23.5%)	2 (66.7%)
Women with SPE	-	0	0	3 (75.0%)	1 (50.0%)

CKD: Chronic kidney disease; CHT: Chronic hypertension; SPE: Superimposed pre-eclampsia

Pregnancy associated subacute kidney injury: >50% rise in Creatinine during pregnancy

Acute kidney injury: >50% rise in Creatinine during pregnancy within one week

GFR: Glomerular filtration rate

Table 1: Longitudinal cohort: Neonatal outcomes for women with pre-eclampsia, healthy controls and women with CKD and/or CHT with and without superimposed pre-eclampsia

	Healthy Controls (N=79)	Pre-eclampsia (N=31)	CKD and/or CHT without superimposed pre-eclampsia (N=124)	CKD and/or CHT with superimposed pre-eclampsia (N=39)
Gestation at delivery (weeks) Median (IQR)	40.1 (39.4, 41.0)*	37.4 (34.6, 38.3)*	38.4 (37.4, 39.6)*	35.6 (33.3, 38.4)*
Delivery <34 weeks	0**	6 (19.4%)**	7 (5.6%)*	14 (35.9%)*
Delivery <37 weeks	3 (3.8%)*	13 (41.9%)*	22 (17.7%)*	24 (61.5%)*
Intrauterine Death	0	1 (3.1%)	1 (0.8%)	1 (2.5%)
Apgar <7 at 1 min	3 (3.8%)*	11 (36.7%)*	6 (5.0%)$^{\$}$	9 (23.7%)$^{\$}$
Apgar <7 at 5 min	1 (1.3%)	1 (3.3%)	0	2 (5.3%)
Birth weight (g) Median (IQR)	3460 (3160, 3760)*	2400 (1670, 3230)*	3010 (2552, 3245)*	2300 (1580, 2775)*
Birth weight centile Median (IQR)	49 (29, 69)*	14 (0, 29)*	28.5 (9.2, 56.7)*	13 (1, 44)*
Small for Gestational Age < 3rd Centile	0*	10 (31.3%)*	18 (14.4%)$^{\$\$}$	12 (30.0%)$^{\$\$}$
Small for Gestational Age < 5th Centile	4 (5.1%)*	12 (38.7%)*	23 (18.5%)$^{\#}$	15 (38.5%)$^{\#}$
Baby transferred to NICU or SCBU	2 (2.6%)*	16 (51.6%)*	11 (8.9%)*	15 (38.5%)*

CKD: Chronic kidney disease; CHT: Chronic hypertension; NICU: Neonatal intensive care unit; SCBU: Special care baby unit

For analyses of neonatal outcomes other than intrauterine death, infants with intrauterine death were excluded

Comparisons are between a) healthy control women and pre-eclampsia b) women with CKD and/or CHT with and without superimposed pre-eclampsia

*P<0.0001; **P=0.0004; $^{\$}$P=0.0018; $^{\$\$}$P=0.035; $^{\#}$P=0.018

Table 5: Longitudinal cohort: Test performance statistics for low PlGF as a prognostic indicator at time of sampling for subsequent delivery within 14 days for superimposed pre-eclampsia at 20^{+0}-36^{+6} weeks and 20^{+0}-40^{+6} weeks in women with chronic kidney disease and/or chronic hypertension

	PlGF <5th Centile for gestation	PlGF <12 pg/ml	PlGF <100 pg/ml
20^{+0}-36^{+6} weeks			
Sensitivity % (95% CI)	75.0 (42.8-94.5)	50.0 (21.1-78.9)	75.0 (42.8-94.5)
n/N	9/12	6/12	9/12
Specificity % (95% CI)	77.5 (68.6-84.9)	95.5 (89.8-98.5)	73.9 (64.8-81.7)
n/N	86/111	106/111	82/111
Positive Predictive Value % (95% CI)	26.47 (12.9-44.4)	54.5 (23.4-83.2)	23.7 (11.4-40.2)
n/N	9/35	6/11	9/38
Negative Predictive Value (95% CI)	96.6 (90.5-99.3)	94.6 (88.7-98.0)	96.5 (90.0-99.3)
n/N	86/89	106/112	82/85
Positive likelihood ratio (95% CI)	3.33 (2.17-5.36)	11.10 (3.98-30.99)	2.87 (1.83-4.51)
Negative likelihood ratio (95% CI)	0.32 (0.12-0.86)	0.52 (0.30-0.92)	0.34 (0.13-0.91)
ROC (SE)	0.85 (0.06)	-	-
20^{+0}-40^{+6} weeks			
Sensitivity % (95% CI)	68.7 (41.3-89.0)	43.7 (19.7-70.1)	81.2 (54.3-95.9)
n/N	11/16	7/16	13/16
Specificity % (95% CI)	81.06 (73.3-87.3)	96.2 (91.4-98.8)	73.5 (65.1-80.8)
n/N	107/132	127/132	97/132
Positive Predictive Value % (95% CI)	30.6 (16.3-48.1)	58.3 (27.7-84.8)	27.1 (15.3-41.8)
n/N	11/36	7/12	13/48
Negative Predictive Value % (95% CI)	95.5 (89.9-98.5)	93.4 (87.8-96.9)	97.0 (91.5-99.4)
n/N	107/112	127/136	97/100
Positive likelihood ratio (95 CI)	3.63 (2.24-5.89)	11.55 (4.15-32.15)	3.06 (2.12-4.43)
Negative likelihood ratio (95 CI)	0.39 (0.19-0.80)	0.58 (0.38-0.90)	0.26 (0.09-0.71)
ROC (SE)	0.83 (0.08)	-	-

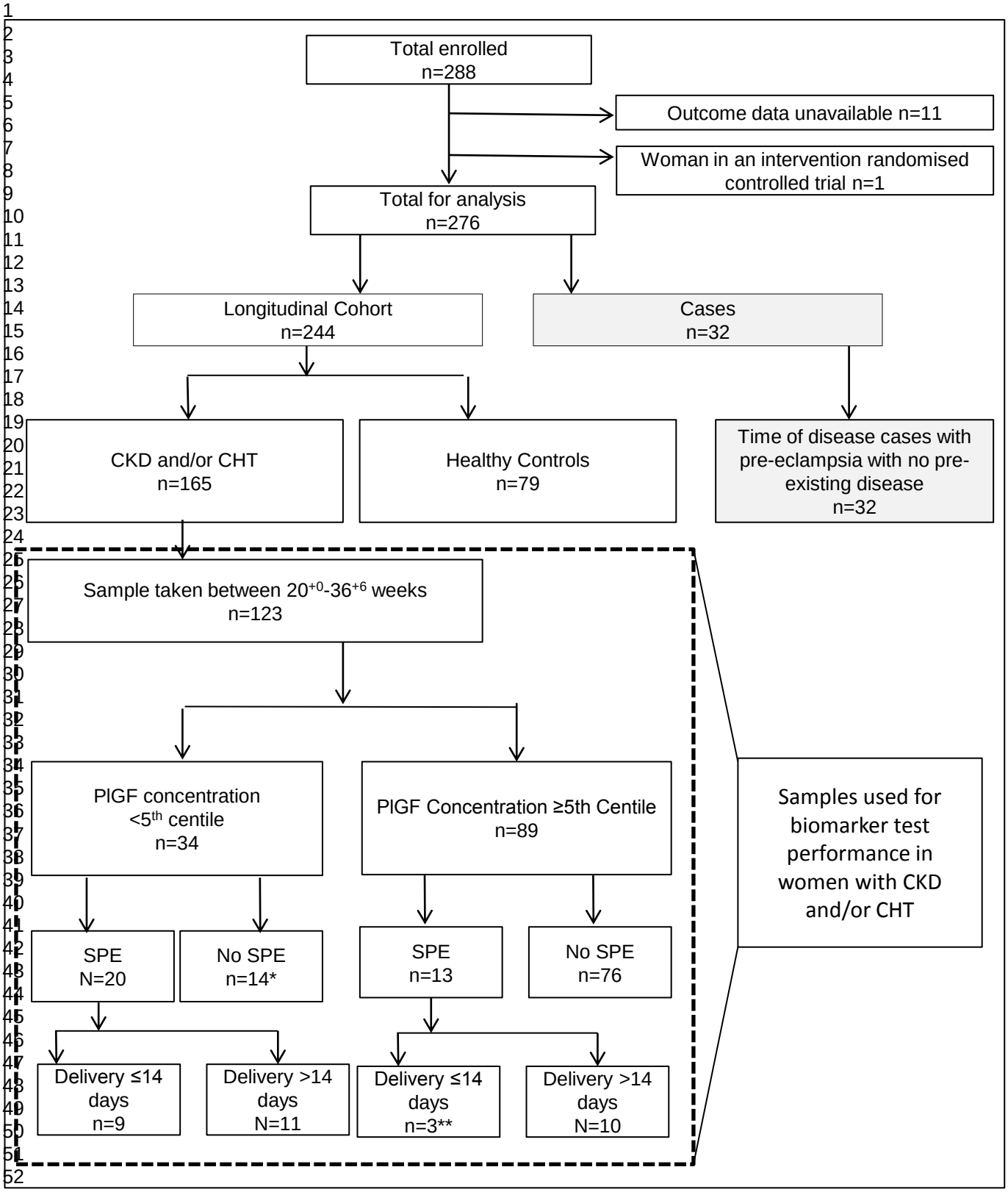
CKD: Chronic kidney disease; CHT: Chronic hypertension

Table 6: Validation cohort: test performance statistics for PlGF <5th centile for gestation as a prognostic indicators at time of sampling for subsequent delivery within 14 days for pre-eclampsia or superimposed pre-eclampsia in women with chronic hypertension, chronic kidney disease or no pre-existing disease (20^{+0}-36^{+6} weeks and 20^{+0}-40^{+6}).

	No pre-existing Disease	CHT or CKD	CHT	CKD
20^{+0}-36^{+6} weeks				
Sensitivity % (95% CI)	87.4 (79.4-93.1)	78.9 (54.4-93.9)	90.9 (58.7-99.8)	62.5 (24.5-91.5)
n/N	90/103	15/19	10/11	5/8
Specificity % (95% CI)	54.8 (47.4-62.1)	72.0 (60.4-81.8)	71.2 (57.9-82.2)	75.0 (47.6-92.7)
n/N	102/186	54/74	42/59	12/15
Positive Predictive Value % (95% CI)	51.7 (44.0-59.4)	41.7 (25.5-59.2)	37.0 (19.4-57.6)	55.6 (21.2-86.3)
n/N	90/174	15/36	10/27	5/9
Negative Predictive Value % (95% CI)	88.7 (81.4-93.8)	93.1 (83.3-98.1)	97.7 (87.7-99.9)	80.0 (51.9-95.7)
n/N	102/115	54/59	42/43	12/16
Positive likelihood ratio (95% CI)	1.93 (1.62-2.30)	2.82 (1.83-4.34)	3.16 (2.03-4.91)	2.50 (0.92-6.82)
Negative likelihood ratio (95% CI)	0.23 (0.14-0.39)	0.29 (0.12-0.71)	0.13 (0.02-0.83)	0.50 (0.20-1.28)
ROC (SE)	0.84 (0.02)	0.82 (0.06)	0.86 (0.05)	0.79 (0.12)
20^{+0}-40^{+6} weeks				
Sensitivity % (95% CI)	74.4 (67.3-80.7)	75.0 (55.1-89.3)	83.3 (58.6-96.4)	60.0 (26.2-87.8)
n/N	131/176	21/28	15/18	6/10
Specificity % (95% CI)	60.7(54.7-66.5)	75.8 (65.9-84.0)	75.0 (63.7-84.2)	78.9 (54.4-93.9)
n/N	170/280	72/95	57/76	15/19
Positive Predictive Value % (95% CI)	54.4 (47.8-60.8)	47.7 (32.5 63.3)	44.1 (27.2-62.1)	60.0 (26.2-87.8)
n/N	131/241	21/44	15/34	6/10
Negative Predictive Value % (95% CI)	79.1 (73.0-84.3)	91.1 (82.6-96.4)	95.0 (86.1-99.0)	78.9 (54.4-93.9)
n/N	170/215	72/79	57/60	15/19
Positive likelihood ratio (95% CI)	1.89 (1.60-2.24)	3.10 (2.05-4.69)	3.33 (2.15-5.18)	2.85 (1.04-7.80)
Negative likelihood ratio (95% CI)	0.42 (0.32-0.55)	0.33 (0.17-0.63)	0.22 (0.08-0.63)	0.51 (0.23-1.12)
ROC (SE)	0.79 (0.02)	0.79 (0.05)	0.82 (0.05)	0.75 (0.12)

CKD: Chronic kidney disease; CHT: Chronic hypertension

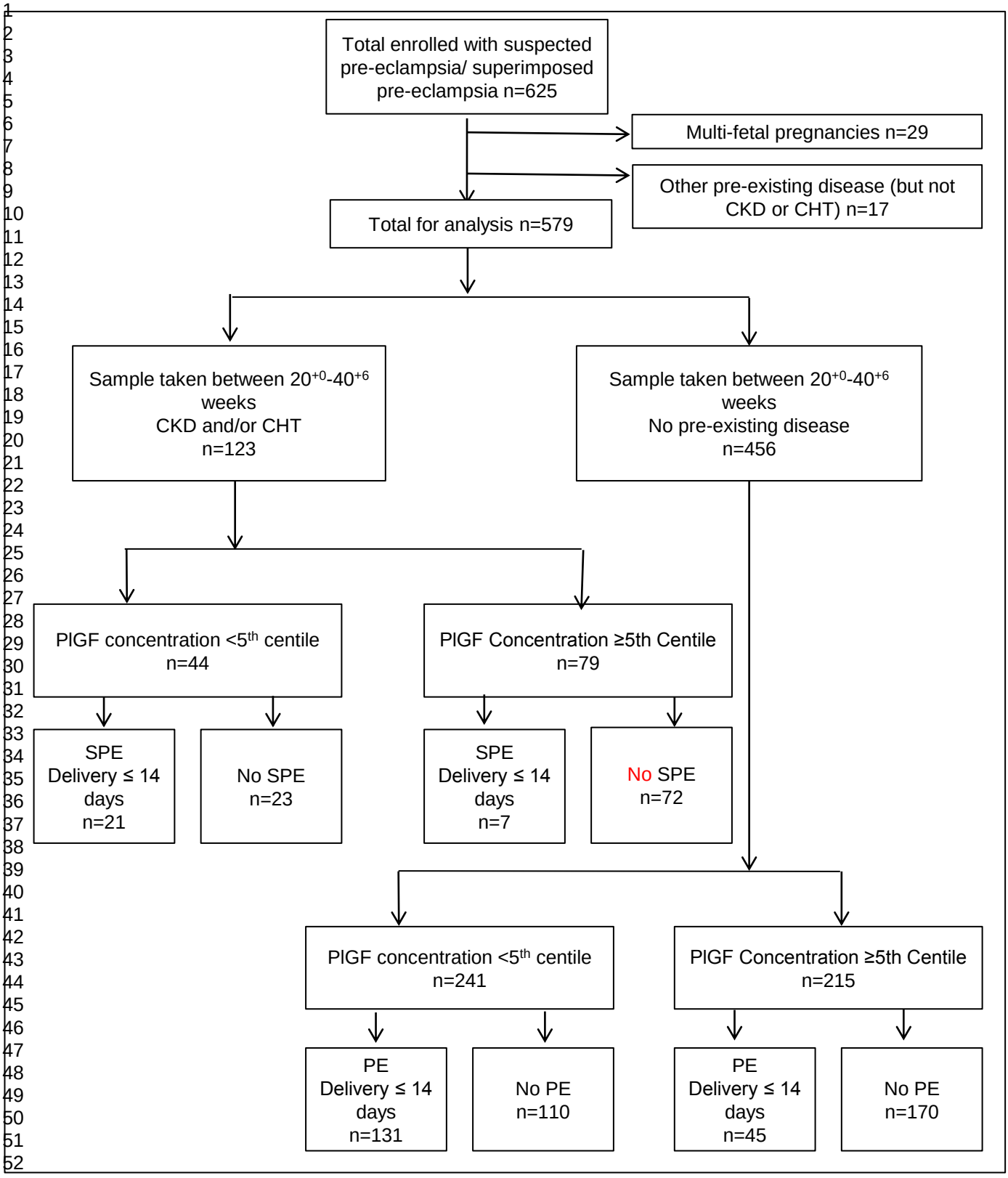
Figure 1a: Longitudinal Cohort and Case-Control Flow Diagram of participants



*Including
- 3 samples taken <22 weeks' gestation
- 8 women with clinical features of SPE but did not meet study criteria for diagnosis
- 3 women with uncomplicated outcomes

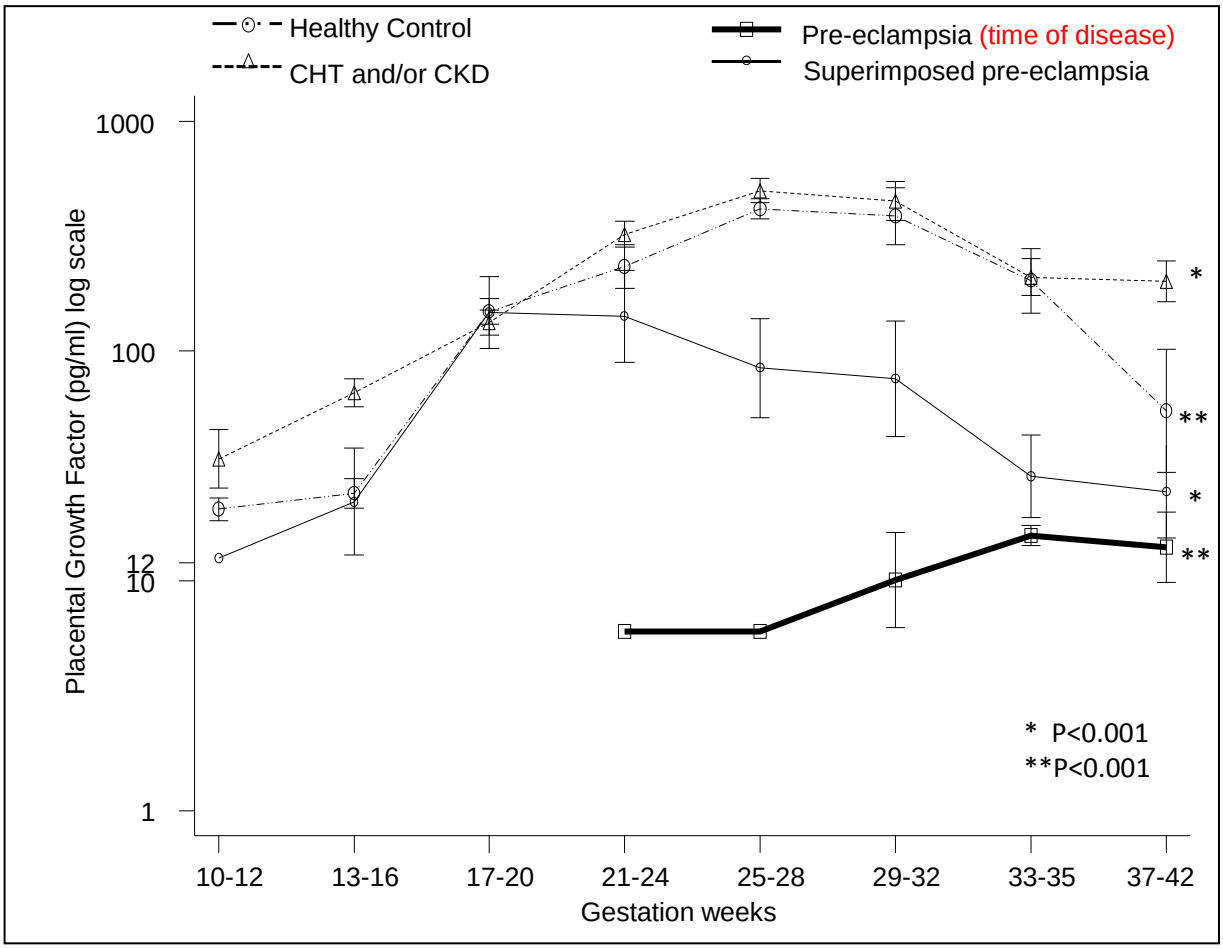
**Including
2 women who met study criteria for diagnosis but clinical suspicion was low
1 woman with catastrophic antiphospholipid syndrome

Figure 1b: Validation Cohort – Flow Diagram of participants



SPE: Superimposed pre-eclampsia; PE: Pre-eclampsia; PlGF: Placental Growth Factor; CKD: Chronic kidney disease; CHT: Chronic Hypertension

Figure 2: Maternal plasma placental growth factor concentrations in healthy controls, women with CHT and/or CKD with and without superimposed pre-eclampsia in a longitudinal cohort and in women with time of disease pre-eclampsia, according to gestation in weeks



Number of Samples								
Healthy Controls	41	12	27	8	7	15	21	5
CHT/CKD	11	28	37	34	36	41	37	36
Superimposed Pre-eclampsia	1	4	6	9	9	13	12	4
Pre-eclampsia	-	-	-	2	2	5	14	9

Mean plasma PlGF concentrations according to gestation in weeks. Bars represent standard errors. Samples were taken from women with SPE before and at time of disease onset. Samples from women with pre-eclampsia were taken after diagnosis. P values are given are for the comparisons between overall PlGF concentration women after logarithmic transformation in samples from women with CHT and/or CKD with and without superimposed pre-eclampsia (*), and healthy controls compared with women with pre-eclampsia without pre-existing disease (**).

CKD: Chronic kidney disease; CHT: Chronic hypertension

Web extra material

Supplementary Table 1: Definitions for study entry

Definition	Criteria
Healthy control women	• No risk factors for pre-eclampsia • No history of pre-eclampsia, hypertension, diabetes, renal disease, connective tissue disease or anti-phospholipid antibody syndrome • Systolic blood pressure <140mmHg • Diastolic blood pressure <90mmHg • No protein on dipstick analysis of midstream urine • Not in labour
Gestational Hypertension	• Previously normotensive • Two recordings of systolic blood pressure ≥140mmHg or diastolic blood pressure ≥ 90mmHg greater than 4 hours apart • After 20 weeks' gestation • Not in labour
Pre-eclampsia	• Gestational Hypertension AND • Proteinuria of >300mg protein over 24 hours, (or protein:creatinine ratio of >30mg/mmol);
Superimposed pre-eclampsia *Hypertension already present*	• New onset of proteinuria >300mg protein over 24 hours, (or protein:creatinine ratio of >30mg/mmol); OR Additional features – severe persistent right upper quadrant pain or epigastric pain unresponsive to mediation or alanine transaminase < 71U/l or platelet count <100,000/µl or pulmonary oedema or new onset cerebral or visual disturbance
Superimposed pre-eclampsia *Proteinuria already present*	• Two recordings of systolic blood pressure ≥140mmHg or diastolic blood pressure ≥ 90mmHg greater than 4 hours apart OR • Additional features as listed above
Superimposed pre-eclampsia *Hypertension and proteinuria already present*	• Development of severe hypertension (Systolic blood pressure ≥160mmHg or diastolic blood pressure ≥110mmHg) AND • Greater than two fold increase in proteinuria above 300mg protein over 24 hours, (or protein:creatinine ratio of >30 mg/mmol); OR Additional features as listed above
Primary Hypertension	• Maternal diastolic blood pressure of 90mmHg or more before 20 weeks' gestation in the current pregnancy OR • Taking antihypertensive agents before 20 weeks' gestation OR • Taking antihypertensives prior to pregnancy • Secondary causes of hypertension excluded
Chronic Hypertension	• Primary or secondary causes of hypertension
Chronic Kidney Disease	• According to Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines pre-pregnancy{Guidelines, #50302} OR • Persistent proteinuria (>1+ or 30mg/mmol (protein creatinine ratio) before 20 weeks' gestation OR • Any recorded serum creatinine >70µmol before 20 weeks' gestation without risk factors for acute kidney injury;
Exclusion	• Women < 18 years old or >50 years old • Inability or unwillingness to give informed consent • Known HIV, Hepatitis B or C positive • Multi-fetal Pregnancy

Supplementary Table 2: Longitudinal cohort: disease aetiology in women with chronic hypertension and/or chronic kidney disease according to development of superimposed pre-eclampsia

Characteristic	CHT and/or CKD without superimposed pre-eclampsia (N=125)	CHT and/or CKD with superimposed pre-eclampsia (N=40)
Primary hypertension	27 (21.6%)*	17 (42.5%)*
Chronic kidney disease only	53 (42.4%)**	8 (20.0%)**
Chronic kidney disease with hypertension	45 (36.0%)	15 (37.5%)
Definition of CHT	N=72	N=32
Pre pregnancy anti-hypertensives	58 (80.5%)	24 (75.0%)
Antihypertensive <20 weeks	54 (75.0%)	22 (68.8%)
DBP >90 mmHg <20 weeks	29 (40.3%)	15 (46.9%)
CKD Stage	N=98	N=23
1	46 (47%)	10 (43.5%)
2	27 (28%)	4 (17.4%)
3	21 (21%)	6 (26.1%)
4	3 (3.1%)	3 (13.0%)
5	1 (1.0%)	-
≥2+ Proteinuria at booking	N=122 32 (26.2%)	N=40 7 (17.5%)
CKD Diagnosed during pregnancy	N=98 14 (14.3%)	N=23 5 (21.7%)
Biopsy proven CKD	N=97 45 (46.4%)	N=23 9 (39.1%)
Cause of CKD		
Adult Polycystic kidney disease	4 (4.1%)	2 (8.7%)
Reflux	8 (8.2%)	2 (8.7%)
Lupus	22 (22.4%)	5 (21.7%)
Immunoglobulin A nephropathy	10 (10.2%)	1 (4.3%)
Minimal Change	3 (3.1%)	0
Focal Segmental Glomerulosclerosis	7 (7.1%)	1 (4.3%)
Vasculitits	1 (1.0%)	0
Interstitial	4 (4.1%)	1 (4.3%)
Transplant	8 (8.2%)	1 (4.3%)
Simultaneous Pancreas Kidney Transplant	2 (2.0%)	2 (8.7%)
Hypertensive Nephropathy	3 (3.1%)	0
Diabetic Nephropathy	1 (1.0%)	1 (4.3%)
Multiple Calculi with impaired function	3 (3.1%)	0
Single Kidney	2 (2.0%)	1 (4.3%)
End Stage	1 (1.0%)	0
Undiagnosed	19 (19.4%)	6 (26.1%)

*p=0.013; ** p=0,0014

CHT: Chronic Hypertension; CKD: Chronic Kidney Disease

Supplementary Table 3: Longitudinal cohort: demographics of women with primary hypertension and chronic kidney disease according to stage

	CHT N=44	CKD Stage 1 N=56	CKD Stage 2 N=31	CKD Stage 3 N=27	CKD Stage 4 or 5 N=7
Age Category (years)					
<20	0	0	1 (3.2%)	0	0
20-29	11 (25%)	24 (42.9%)	8 (25.8%)	6 (18.5%)	1 (14.3%)
30-39	22 (50%)	31 (55.4%)	18 (58.1%)	18 (66.7%)	6 (85.7%)
≥40	11 (25%)	1 (1.8%)	4 (12.9%)	4 (14.8%)	0
BMI Category (kg/m^2)					
<20	0	8 (14/3%)	2 (6.5%)	2 (7.4%)	0
20-24	10 (22.7%)	20 (35.7%)	12 (38.7%)	13 (48.1%)	3 (42.9%)
25-29	12 (27.3%)	11 (19.6%)	9 (29.0%)	8 (29.6%)	3 (42.9%)
30-34	11 (25.0%)	10 (17.9%)	5 (16.1%)	2 (7.4%)	1 (14.3%)
35-39	8 (18.2%)	4 (7.1%)	2 (6.5%)	2 (7.4%)	0
≥40	3 (6.8%)	3 (5.4%)	1 (3.2%)	0	0
Ethnicity					
White	17 (38.6%)	21 (37.5%)	14 (45.2%)	19 (70.4%)	2 (28.6%)
Black	25 (56.8%)*	21 (37.5%)	9 (29.0%)	4 (14.8%)*	4 (57.1%)
Asian	1 (2.3%)	6 (10.7%)	6 (19.4%)	2 (7.4%)	0
Other	1 (2.3%)	8 (14.3%)	2 (7.1%)	2 (7.4%)	1 (14.3%)
Smoking					
Never	39 (88.6%)	51 (91.1%)	29 (93.5%)	24 (88.9%)	7 (100%)
Stopped before pregnancy	1 (2.3%)	2 (3.5%)	1 (3.2%)	0	0
Current	4 (9.1%)	3 (5.4%)	1 (3.2%)	3 (11.1%)	0
Nulliparous	16 (36.4%)	31 (55.4%)	16 (51.6%)	14 (51.9%)	2 (28.6%)
One or more pregnancy losses					
≤12 weeks	9 (20.5%)	17 (30.4%)	6 (19.4%)	8 (29.6%)	1 (14.3%)
13-24 weeks	3 (6.8%)	6 (10.7%)	3 (9.7%)	3 (11.1%)	1 (14.3%)
≥24 weeks	4 (9.1%)	5 (8.9%)	2 (6.5%)	1 (3.7%)	0
≥2+ Proteinuria at booking	N=43 0	N=55 21 (38.2%)	N=31 4 (12.9%)	N=27 11 (40.7%)	N=6 3 (50.0%)
Chronic hypertension	44 (100%)	21 (37.5%)	15 (48.4%)	18 (66.7%)**	5 (71.4%)
Assisted Conception	0	2 (3.6%) N=41	2 (6.5%) N=25	3 (11.1%) N=23	0 N=7
Median Pre-pregnancy eGFR (IQR) mls/min/1.73m^2	-	108 (103, 123)	66 (76, 82)	49 (40, 54)	23 (15, 28)
Median Pre-pregnancy Creatinine µmol/l	-	61 (55, 66)	89 (80, 95)	116 (108, 141)	266 (201, 384)

CHT: Chronic Hypertension; CKD: Chronic Kidney Disease; eGFR: Estimated glomerular filtration rate

*Primary hypertension v CKD Stage 1 or 2 p=0.0015 compared with white ethnicity

** CKD Stage 1 or 2 v Stage 3 p=0.046

Supplementary Table 4: Longitudinal cohort: outcomes according to presence of chronic hypertension or stage of chronic kidney disease

Key outcomes	Primary CHT	CKD stage 1-2	CKD stage 3	CKD stage 4-5	Primary CHT v Stage 1-2 P value	Stage 1-2 v 3 P value	Stage 1-2 v 4-5 P value
Delivery <34 weeks	N=43 3 (7.0%)*	N=86 9 (10.5%)	N=28 4 (14.3%)	N=7 3 (42.9%)	NS	NS	0.043
Delivery <37 weeks	N=43 8 (18.6%)	N=86 20 (23.3%)	N=28 11 (39.3%)	N=7 7 (100.0%)	NS	NS	0.0001
Intrauterine Death	N=43 1 (2.3%)	N=87 1 (1.0%)	N=28 0 (0.0%)	N=7 0 (0.0%)	NS	NS	NS
Gestation at delivery (weeks) Median (IQR)	39.00 (37.79-39.57)	38.12 (37.12-39.00)	37.22 (35.93-38.00)	34.00 (33.29-35.50)	NS	0.006	0.006
Birth weight (g) Median (IQR)	3100 (2580-3475)	2939 (2500-3210)	2490 (2128-2849)	1840 (1830-2173)	NS	0.002	0.002
Birth weight centile Median (IQR)	25.0 (7.0-50.0)	29.0 (13.0-57.0)	12.5 (1.0-40.5)	4.0 (3.5-18.5)	NS	0.01	0.021
Small for Gestational Age < 3rd Centile	N=43 9 (20.9%)	N=86 10 (11.6%)*	N=28 10 (35.7%)*	N=7 2 (28.6%)	NS	0.008	NS
Small for Gestational Age < 5rd Centile	N=43 10 (23.3%)	N=86 14 (16.3%)	N=28 11 (39.3%)	N=7 4 (57.1%)	NS	0.017	0.025
Baby transferred to NICU or SCBU	N=43 4 (9.3%)	N=85 10 (11.8%)	N=28 9 (32.1%)	N=7 4 (57.1%)	NS	0.019	0.009

CHT: Chronic hypertension; CKD: Chronic kidney disease; NICU: Neonatal intensive care unit; SCBU: Special care baby unit; NS: Not significant

Supplementary Table 5: Longitudinal cohort: predictive value of PlGF concentration <5th centile in women with chronic hypertension or chronic kidney disease between women who did and did not develop superimposed pre-eclampsia before disease onset according to gestation, excluding women with diagnosed superimposed pre-eclampsia

Gestation	**21-24^{+6} weeks**	**25-28^{+6} weeks**	**29-32^{+6} weeks**	**33-36^{+6} weeks**
Sensitivity % (95% CI)	25 (3.2-65.1)	66.7 (22.3-95.7)	42.9 (9.9-81.6)	50.0 (6.8-93.2)
n/N	2/8	4/6	3/7	2/4
Specificity % (95% CI)	97.1 (84.7-99.9)	97.1 (85.1-99.9)	87.8 (73.8-95.9)	86.1 (70.5-95.3)
n/N	33/34	34/35	36/41	31/36
Positive Predictive Value % (95% CI)	66.7 (9.4-99.2)	80.0 (28.4-99.5)	37.5 (8.5-75.5)	28.6 (3.7-71.0)
n/N	2/3	4/5	3/8	2/7
Negative Predictive Value % (95% CI)	84.6 (69.5-94.1)	94.4 (81.3-99.3)	90.0 (76.3-97.2)	93.9 (79.8-99.3)
n/N	33/39	34/36	36/40	31/33
Positive likelihood ratio (95% CI)	8.50 (0.88-82.57)	23.33 (3.12-174.65)	3.51 (1.07- 11.50)	3.60 (1.01-12.86)
Negative likelihood ratio (95% CI)	0.77 (0.52-1.16)	0.34 (0.11-1.07)	0.65 (0.34-1.25)	0.58 (0.22-1.56)
ROC (SE)	0.53 (0.14)	0.78 (0.16)	0.64 (0.13)	0.83 (0.09)

Supplementary Table 6: Longitudinal cohort: false positives (PlGF <5th Centile) and false negatives (PlGF≥5th Centile) for women with chronic hypertension or chronic kidney disease for samples taken between 20 and 36+6 weeks' gestation

Subject	CHT or CKD	Gestation Sampling	Gestation Delivery	PlGF (pg/ml)	PlGF Centile	Creatinine (µmol/L)	Birth Weight (g)	Birth Weight Centile	Severe Hypertension	Doubling of Proteinuria over threshold	Details
False Positives											
Clinical suspicion of superimposed pre-eclampsia but did not meet criteria											
21	CKD Stage 1 Lupus nephritis (Unknown class) and CHT	31^{+6}	32^{+0}	56.6	1	66	1950	46	0	1	Induced day after sample taken for fetal growth restriction. Neonatal death at 8 weeks.
41	CKD Stage 1 CHT and previous calculi and hydronephrosis	32^{+0}	38^{+6}	<12	0	51	2650	8	1	0	Elective section for cerebral aneurysm. Poorly controlled blood pressure.
66	CKD Stage 2 ANCA vasculitis	34^{+3}	37^{+1}	41.6	3	78	2550	13	0	0	Clinical suspicion of superimposed pre-eclampsia at 34 weeks' but expectant management until 37 weeks.
79	CKD Stage 3 Class IV Lupus	28^{+0}	30^{+1}	52.6	0	185	1130	1	0	1	Spontaneous preterm labour at 30 weeks with fetal growth restriction.
83	CKD Stage 2 Renal Transplant and CHT	35^{+3}	37^{+2}	24	2	109	3120	73	0	1	Induced for increasing creatinine and falling platelets, but did not meet criteria for superimposed pre-eclampsia
160	CHT only	34^{+4}	39^{+1}	40.3	3	53	3040	14	1	0	Induced for reduced fetal growth Transient proteinuria above threshold
179	CKD Stage 1 Class V lupus	30^{+4}	32^{+5}	36.1	0	84	1890	17	0	1	Induced for reduced fetal growth and cardiac defect; clinical suspicion of superimposed pre-eclampsia but hypertension not over threshold.
180	CKD Stage 1 Class III and IV lupus	20^{+6}	24^{+1}	<12	0	68	280	0	0	0	Active lupus nephritis Intrauterine death of growth restricted fetus at 24 weeks
No clinical suspicion of superimposed pre-eclampsia											
4	CKD Stage 3 Hypertensive Nephropathy	20^{+3}	37^{+6}	57.5	3	84	2220	1	1	1	Early sampling
40	CHT only	30^{+4}	38^{+2}	116	3	45	3350	62	0	0	Spontaneous labour No complications
196	CKD Stage 1 Minimal Change Disease	34^{+2}	40^{+3}	50.3	4	58	2910	7	0	0	Spontaneous labour No complications
285	CHT only	21^{+1}	35^{+4}	36	0	65	1620	0	0	0	Early sampling
289	CKD Stage 3 and CHT Unknown cause	20^{+5}	38^{+3}	55.3	3	75	2095	0	0	0	Early sampling
731	CKD Stage 1 Medullary Sponge Kidney	33^{+0}	40^{+2}	12.4	0	117	3200	21	0	0	Spontaneous labour No complications
False Negatives											
170	CKD Stage 3 Kidney Pancreas Transplant and CHT	30^{+4}	32^{+2}	2630	98	132	1750	20	1	1	Recurrent admissions for suspected superimposed pre-eclampsia with fluctuating creatinine. Elective section for

											increasing creatinine.
252	CKD Stage 1 Type 1 DM with newly diagnosed nephropathy	33^{+0}	34^{+1}	115	9	46	2650	71	1	1	Unclear clinical diagnosis as possible gestational hypertension and but met diagnostic criteria. Induced for fetal decelerations
263	CHT and antiphospholipid syndrome	34^{+2}	34^{+3}	187	30	84	2320	25	1	0	Delivery for HELLP syndrome and suspected fetal growth restriction

CHT: Chronic hypertension; CKD: Chronic Kidney Disease; PlGF: Placental growth factor; Severe Hypertension: Systolic blood pressure ≥160mmHg or Diastolic blood pressure ≥110mmHg; DM: Diabetes Mellitus; HELLP: Haemolysis elevated liver enzymes, low platelets

Supplementary Table 7: Predictive value of uterine and umbilical artery Dopplers in women with chronic hypertension or chronic kidney disease for superimposed pre-eclampsia

	Uterine artery Mean PI >1.4 *20-24^{+6} weeks*	**Uterine artery bilateral notching** *20-24^{+6} weeks*	**Umbilical artery PI >95th Centile** *All studies>28 weeks*	**Umbilical artery PI >95th Centile** *<14 days before delivery*
Sensitivity % (95% CI)	20.00	28.57	7.69	11.11
n/N	(3.1-55.6)	(8.6 -58.1)	(1.3 -36.1)	(1.8 -48.3)
	2/10	4/14	1/13	1/9
Specificity % (95% CI)	100.00	92.59	91.84	92.31
n/N	(78.0 -100.0)	(75.7 -98.8)	(80.3 -97.7)	(63.9 -98.7)
	15/15	25/27	45/49	12/13
Positive Predictive Value % (95% CI)	100.00	66.67	20.00	50.00
n/N	(19.3 -100.0)	(22.7 -94.7)	(3.3 -71.2)	(8.2 -91.8)
	2/2	4/6	1/5	1/2
Negative Predictive Value % (95% CI)	65.22	71.43	78.95	60.00
n/N	(42.7 -83.6)	(53.7 -85.3)	(66.1 -88.6)	(36.1 -80.8)
	15/23	25/35	45/57	12/20
Positive likelihood ratio (95% CI)		3.86	0.94	1.44
		(0.80-18.54)	(0.11-7.73)	(0.10-20.21)
Negative likelihood ratio (95% CI)	0.80	0.77	1.01	0.96
	(0.6-1.1)	(0.5-1.1)	(0.8-1.2)	(0.7-1.3)

Supplementary Table 8: Validation cohort: characteristics at first antenatal visit and enrolment at time suspected pre-eclampsia

Characteristic	No pre-existing Disease N=456	Chronic Hypertension N=94	Chronic Kidney Disease N=29
Age (years)	31.2	33.5	32.7
Median (IQR)	(26.5 to 35.3)	(30.7 to 36.6)	(29.2 to 38.2)
Body Mass Index (kg/m^2)	27.7	31.1	26.3
Median (IQR)	(23.6 to 31.6)	(26.7 to 36.8)	(23.7 to 30.3)
Systolic blood pressure at first antenatal visit (mmHg)	118.0	140.0	120.0
Median (IQR)	(108.3 to 122.0)	(126.8 to 145.0)	(117.0 to 133.5)
Diastolic blood pressure at first antenatal visit (mmHg)	70.0	88.0	75.0
Median (IQR)	(64.0 to 78.0)	(80.0 to 92.0)	(70.0 to 83.0)
Nulliparous	275 (60.3%)	36 (38.3%)	14 (48.2%)
Ethnicity			
White	313 (68.6%)	59 (62.8%)	21 (72.4%)
Black	80 (17.5%)	26 (27.7%)	8 (27..6%)
Asian	36 (7.9%)	5 (5.3%)	0
Other	27 (5.9%)	4 (4.3%)	0
Smoking		N=91	
Never	324 (72.3%)	72 (79.1%)	22 (75.9%)
Ex-smoker	80 (17.9%)	13 (14.3%)	5 (17.2%)
Current	44 (9.8%)	6 (6.6%)	2 (6.9%)
Previous Pre-eclampsia	N=452	N=93	
<34 weeks'	24 (5.3%)	16 (17.2%)	3 (10.7%)
≥34 weeks'	43 (9.5%)	8 (8.6%)	3 (10.7%)
Other disease		N=90	
Antiphospholipid Syndrome or Systemic Lupus Erythematosus	0	1 (1.1%)	3 (10.3%)
Pregestational Diabetes Mellitus	0	4 (4.4%)	2 (6.9%)
Chronic Hypertension	0	94 (100%)	8 (27.6%)
Enrolment/Suspected pre-eclampsia			
Gestation week	35.9	34.1	33.4
Median (IQR)	(32.5 to 37.9)	(27.9 to 37.0)	(30.6 to 36.6)
Signs/symptoms of suspected pre-eclampsia (non-exclusive)			
New onset hypertension	342 (75.0%)	22 (23.4%)	16 (55.1%)
Worsening of underlying hypertension	45 (98.7%)	58 (61.7%)	6 (20.7%)
New onset dipstick proteinuria	260 (57.0%)	46 (48.9%)	18 (62.1%)
Persistent epigastric /right upper quadrant pain	27 (5.9%)	6 (22.2%)	2 (6.9%)
Headaches/Visual Disturbance	157 (34.4%)	30 (31.9%)	8 (27.6%)
Suspected fetal growth restriction	27 (5.9%)	2 (2.1%)	0
Enrolment Laboratory Parameters			
Median (IQR)			
	N=403	N=81	N=23
Alanine Transaminase (U/L)	14.0	14.0	16.0
	(11.0 to 20.0)	(11.0 to 20.0)	(12.0 to 25.0)
	N=433	N=86	N=26
Creatinine (µmol/L)	53.0	55.0	70.5
	(46.0 to 62.0)	(45.0 to 64.0)	(50.8 to 118.0)
	N=321	N=64	N=15
Uric acid (µmol/L)	300.0	264.0	335.0
	(220.0 to 360.0)	(220.8 to 329.8)	(246.0 to 380.0)
	N=438	N=87	N=27
Platelets (x10^9/L)	220.0	243.0	242.0
	(180.0 to 266.0)	(206.0 to 277.0)	(187.0 to 269.0)
Proteinuria	N=407	N=80	N=23
Negative or trace	162 (39.8)	40 (48.8)	4 (17.4)
1	105 (25.8)	25 (30.5)	7 (30.4)
≥2	140 (34.4)	17 (20.7)	12 (52.2)

Supplementary Table 9: Validation cohort: maternal and neonatal outcomes

	No pre-existing Disease N=456	Chronic Hypertension N=94	Chronic Kidney Disease N=29
Maternal Outcomes			
Pre-eclampsia/ Superimposed pre-eclampsia	253 (55.5%)	44	17
Final Diagnosis	N=253		
Mild pre-eclampsia	82 (32.4%)	-	-
Severe pre-eclampsia	110 (43.5%)	-	-
Atypical pre-eclampsia	58 (22.9%)	-	-
Eclampsia	1 (0.4%)	0	0
HELLP Syndrome	2 (0.8%)	0	0
Gestational Hypertension	79 (31.2%)	0	1 (3.4%)
Isolated Proteinuria	21 (8.3%)	0	4 (13.8%)
Isolated SGA (<10th Customised birthweight centile)	12 (4.7%)	0	0
Transient Hypertension	50 (19.8%)	0	2 (6.9%)
Normal	37 (14.6%)	0	0
Other	4 (1.6%)	0	1 (3.4%)
Adverse maternal parameters			
Highest systolic (mmHg) Median (IQR)	143.0 (131.0 to 155.0)	150.0 (140.0 to 160.0)	140.0 (129.0 to 150.5)
Highest diastolic (mmHg) Median (IQR)	92.0 (84.0 to 100.0)	97.5 (90.0 to 102.0)	83.0 (79.0 to 94.5)
Severe hypertension (SBP>160)	115 (25.2%)	30 (6.6%)	5 (17.2%)
Alanine aminotransferase >70 iu/L	28 (6.1%)	5 (5.3%)	2 (6.9%)
Platelets<50 x10^{9}/l	1 (0.2%)	0	0
Antihypertensive Use			
1 drug	91 (20.0%)	22 (23.4%)	5 (17.2%)
2 drugs	38 (8.3%)	23 (24.5%)	5 (17.2%)
≥ 3 drugs	15 (3.3%)	9 (9.6%)	4 (13.8%)
Required IV Magnesium Sulphate	7 (1.5%)	1 (1.0%)	1 (3.4%)
Onset of labour	N=454	N=92	N=29
Spontaneous	103 (22.7%)	12 (13.0%)	4 (13.8%)
Induction of labour	221 (48.7%)	45 (48.9%)	14 (48.3%)
Prelabour Caesarean Section	130 (28.6%)	35 (38.0%)	11 (37.9%)
Mode of delivery	N=434	N=93	N=28
Spontaneous vaginal	171 (39.4%)	35 (37.6%)	7 (25.0%)
Caesarean	209 (48.2%)	49 (52.7%)	17 (60.7%)
Assisted vaginal	54 (12.4%)	9 (9.7%)	4 (14.3%)
Proteinuria			
No proteinuria	175 (38.4%)	42 (44.7%)	7 (24.1%)
Dipstick Proteinuria Present (1+ or greater)	120 (26.3%)	26 (27.7%)	8 (27.6%)
Protein: Creatinine Ratio > 30mg/mmol	87 (19.1%)	9 (9.6%)	8 (27.6%)
24 hour urine >300mg	74 (16.2%)	17 (18.1)	6 (20.7%)
Neonatal Outcomes			
Perinatal Death	6 (1.3%)	2 (2.1%)	1 (3.4%)
Gestation at delivery (weeks) Median (IQR)	38.4 (36.9 to 39.7)	38.1 (36.2 to 40.0)	37.0 (35.9 to 38.3)
Preterm <37 weeks'	118 (25.9%)	32 (34.0%)	13 (44.8%)
Preterm <34 weeks'	51 (11.2%)	12 (12.8%)	5 (17.2%)
Birth Weight (g) Median (IQR)	3053 (2303 to 3438)	3035 (2500 to 3398)	2500 (2260 to 3125)
SGA (<10th customised birth weight centile)	161 (35.3%)	35 (37.2%)	11 (37.9%)
SGA (<3rd customised birth weight centile)	112 (24.6%)	18 (19.1%)	6 (20.7%)

HELLP: Haemolysis, elevated liver enzymes and low platelets

Supplementary Table 10: Validation cohort: Median and interquartile ranges for low PlGF, sFlt-1 and sFlt-1:PlGF for women with chronic hypertension, chronic kidney disease and no pre-existing disease at 20^{+0}-33^{+6} weeks', 20^{+0}-36^{+6} weeks', and 20^{+0}-40^{+6} weeks' gestation

	No pre-existing Disease Pre-eclampsia	No pre-existing Disease No Pre-eclampsia	Chronic Hypertension Pre-eclampsia	Chronic Hypertension No Pre-eclampsia	Chronic Kidney Disease Pre-eclampsia	Chronic Kidney Disease No Pre-eclampsia
20^{+0}-33^{+6}	N=49	N=106	N=5	N=41	N=5	N=10
PlGF pg/ml	10.0 (10.0,14.1)	152.8 (42.6,362.7)	10.0 (10.0,10.0)	282.2 (88.9,553.2)	10.0 (10.0,24.2)	207.7 (87.5,449.4)
sFlt-1 pg/ml	5.2 (3.0,9.6)	0.9 (0.6,1.8)	4.5 (1.2,5.8)	0.9 (0.5,1.8)	4.3 (3.0,4.5)	0.9 (0.5,2.2)
sFlt-1 :PlGF	44.4 (22.1,95.5)	0.6 (0.1,3.8)	45.3 (10.0,58.4)	0.3 (0.1,2.1)	30.0 (25.3,43.0)	0.4 (0.1,2.6)
20^{+0}-36^{+6}	N=103	N=186	N=11	N=59	N=8	N=16
PlGF pg/ml	10.0 (10.0,24.2)	97.2 (26.8,296.9)	12.9 (10.0,28.2)	214.0 (46.9,414.6)	21.2 (10.0,54.3)	234.9 (59.7,372.6)
sFlt-1 pg/ml	5.4 (2.9,8.0)	1.3 (0.7,3.4)	4.5 (1.2,6.1)	1.1 (0.5,2.3)	4.4 (3.0,5.4)	0.9 (0.5,2.8)
sFlt-1 :PlGF	33.0 (16.0,67.5)	1.3 (0.3,11.1)	27.2 (10.0,58.4)	0.5 (0.1,8.0)	27.7 (7.1,37.6)	0.3 (0.1,5.7)
20^{+0}-40^{+6}	N=176	N=279	N=18	N=76	N=10	N=19
PlGF pg/ml	14.5 (10.0,29.0)	60.0 (23.2,246.0)	13.3 (10.0,28.2)	116.7 (34.6,327.7)	21.2 (10.0,61.0)	209.4 (65.7,295.8)
sFlt-1 pg/ml	5.1 (2.8,7.2)	1.8 (0.9,3.9)	4.0 (2.3,6.1)	1.4 (0.6,2.5)	3.7 (2.4,4.9)	1.1 (0.5,3.4)
sFlt-1 :PlGF	29.7 (12.1,56.9)	3.0 (0.3,15.1)	25.1 (10.0,53.1)	1.2 (0.2,8.5)	21.6 (6.2,32.3)	0.4 (0.1,6.9)

CKD: Chronic kidney disease; CHT: Chronic hypertension; PlGF: Placental Growth Factor; sFlt-1: soluble fms like tyrosine kinase receptor-1

Supplementary Table 11: Validation cohort: Receiver Operator Curve Areas for women with chronic hypertension, chronic kidney disease and no pre-existing disease at 20^{+0}-33^{+6} weeks', 20^{+0}-36^{+6} weeks', and 20^{+0}-40^{+6} weeks' gestation for low PlGF, sFlt-1 and sFlt-1:PlGF as a prognostic indicators at time of sampling for subsequent delivery within 14 days for superimposed pre-eclampsia.

Sampling gestation (weeks)	**20^{+0}-33^{+6}**	**20^{+0}-33^{+6}**	**20^{+0}-36^{+6}**	**20^{+0}-36^{+6}**	**20^{+0}-40^{+6}**	**20^{+0}-40^{+6}**
	CKD or CHT	**No pre-existing disease**	**CKD or CHT**	**No pre-existing disease**	**CKD or CHT**	**No pre-existing disease**
Number of women (Cases)	61 (14)	155 (49)	94 (25)	289 (103)	123 (61)	456 (253)
ROC (SE) for PlGF	0.84 (0.09)	0.91 (0.02)	0.82 (0.06)	0.84 (0.02)	0.79 (0.05)	0.79 (0.02)
ROC (SE) for sFlt-1	0.79 (0.09)	0.88 (0.02)	0.79 (0.06)	0.81 (0.02)	0.77 (0.04)	0.77 (0.02)
ROC (SE) for sFlt-1:PlGF	0.84 (0.09)	0.91 (0.02)	0.83 (0.06)	0.86 (0.02)	0.80 (0.05)	0.81 (0.02)

ROC: Receiver Operator Curve; SE: Standard Error; CKD: Chronic kidney disease; CHT: Chronic hypertension; PlGF: Placental Growth Factor; sFlt-1: soluble fms like tyrosine kinase receptor-1

Supplementary Table 12: Validation cohort: Test performance statistics for sFlt-1:PlGF Ratio >85 as a prognostic indicator at time of sampling for subsequent delivery within 14 days for superimposed pre-eclampsia at 20^{+0}-36^{+6} weeks and 20^{+0}-40^{+6} weeks in women with chronic kidney disease and/or chronic hypertension

sFlt-1:PlGF >85	No pre-existing Disease N=456	Chronic Hypertension N=94	Chronic Kidney Disease N=29
20^{+0}-33^{+6} weeks			
Sensitivity %	91.8	100.0	90.0
(95% CI)	(80.4-97.7)	(47.8-100.0)	(55.5-99.7)
n/N	45/49	5/5	9/10
Specificity %	81.1	80.5	80.4
(95% CI)	(72.4- 88.1)	(65.1-91.2)	(66.9-90.2)
n/N	86/106	33/41	41/51
Positive Predictive Value % (95% CI)	69.2	38.5	47.4
n/N	(56.6-80.1)	(13.9-68.4)	(24.4-71.1)
	45/65	5/13	9/19
Negative Predictive Value % (95% CI)	95.6	100.0	97.6
n/N	(89.0-98.8)	(89.4-100.0)	(87.4-99.9)
	86/90	33/33	41/42
Positive likelihood ratio	4.87	5.13	4.59
(95% CI)	(3.25-7.29)	(2.75-9.54)	(2.54-8.30)
Negative likelihood ratio	0.10	0.00	0.12
(95% CI)	(0.04-0.26)	-	(0.02-0.80)
20^{+0}-36^{+6} weeks			
Sensitivity % (95% CI)	85.4	90.9	78.9
n/N	(77.1- 91.6)	(58.7- 99.8)	(54.4-93.9)
	88/103	10/11	15/19
Specificity % (95% CI)	71.5	76.3	76.0
n/N	(64.4-77.9)	(63.4-86.4)	(64.7-85.1)
	133/186	45/59	57/75
Positive Predictive Value % (95% CI)	62.4	41.7	45.5
n/N	(53.9-70.4)	(22.1-63.4)	(28.1-63.6)
	88/141	10/24	15/33
Negative Predictive Value % (95% CI)	89.9	97.8	93.4
n/N	(83.8-94.2)	(88.5-99.9)	(84.1-98.2)
	133/148	45/46	57/61
Positive likelihood ratio (95% CI)	3.00	3.83	3.29
	(2.36-3.82)	(2.34-6.28)	(2.07-5.24)
Negative likelihood ratio (95% CI)	0.20	0.12	0.28
	(0.13-0.33)	(0.02-0.78)	(0.11-0.67)
20^{+0}-40^{+6} weeks			
Sensitivity % (95% CI)	83.5	83.3	75.0
n/N	(77.2-88.7)	(58.6-96.4)	(55.1-89.3)
	147/176	15/18	21/28
Specificity % (95% CI)	65.2	73.7	74.7
n/N	(59.3-70.8)	(62.3-83.1)	(64.8-83.1)
	182/279	56/76	71/85
Positive Predictive Value % (95% CI)	60.2	42.9	46.7
n/N	(53.8-66.4)	(26.3-60.6)	(31.7-62.1)
	147/244	15/35	21/45
Negative Predictive Value % (95% CI)	86.3	94.9	91.0
n/N	(80.9-90.6)	(85.9-98.9)	(82.4-96.3)
	182/211	56/59	71/78
Positive likelihood ratio (95% CI)	2.40	3.17	2.97
	(2.02-2.86)	(2.06-4.86)	(1.98-4.46)
Negative likelihood ratio (95% CI)	0.25	0.23	0.33
	(0.18-0.36)	(0.08-0.64)	(0.17-0.64)